CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

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Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1934. Published at 500 So. 5th St., Minneapolis, Minn. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

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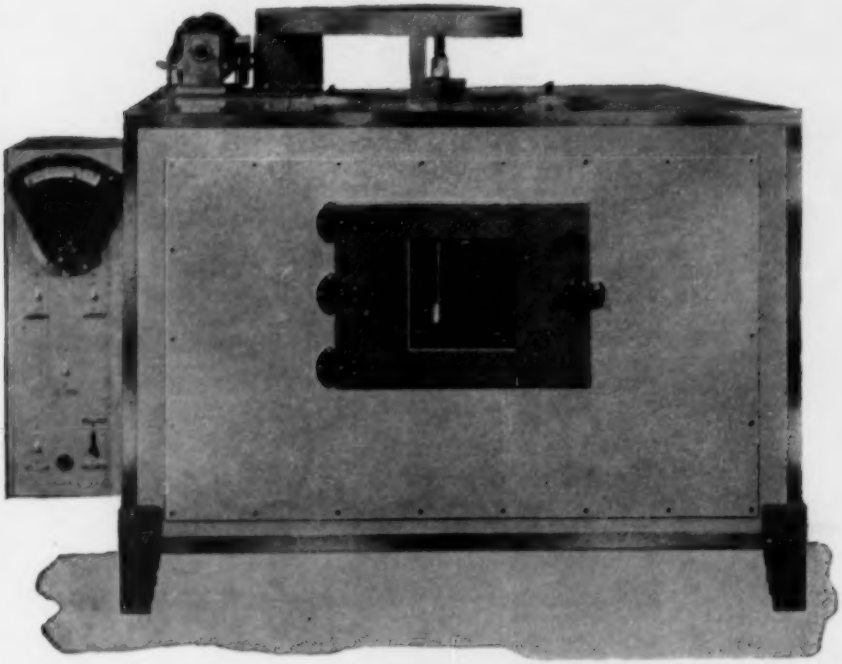

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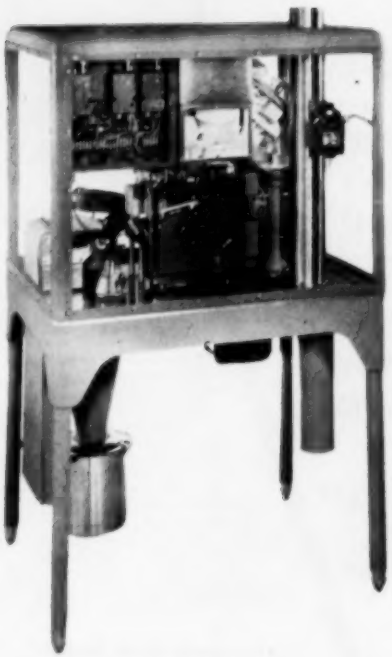

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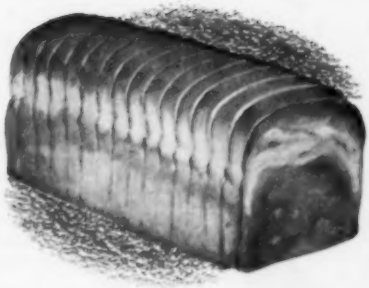

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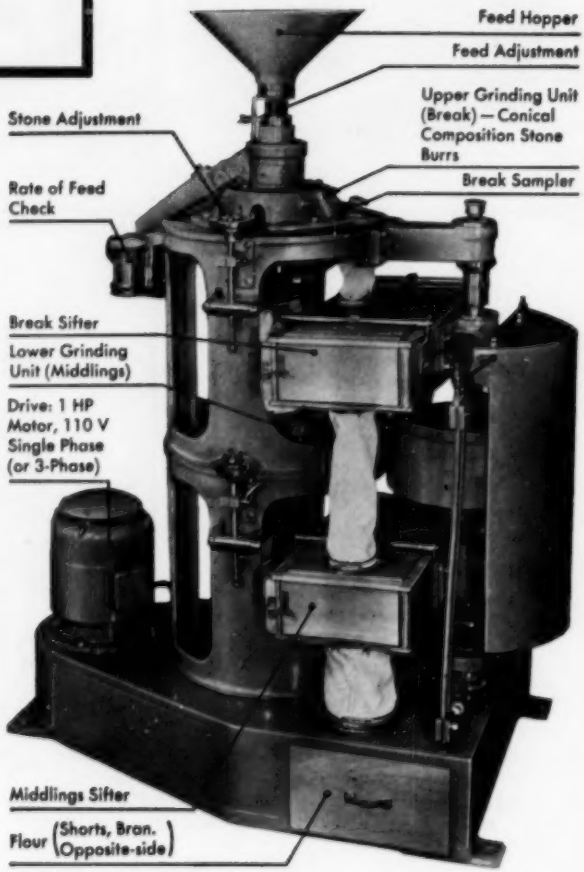

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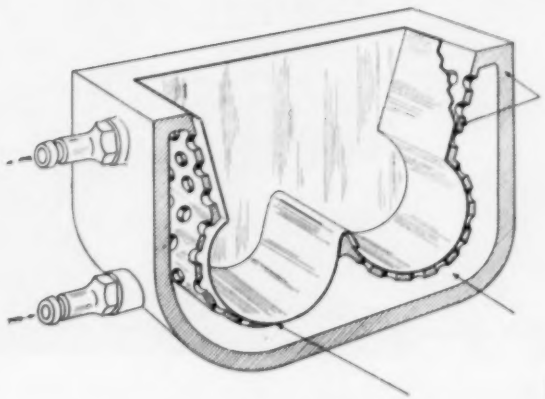

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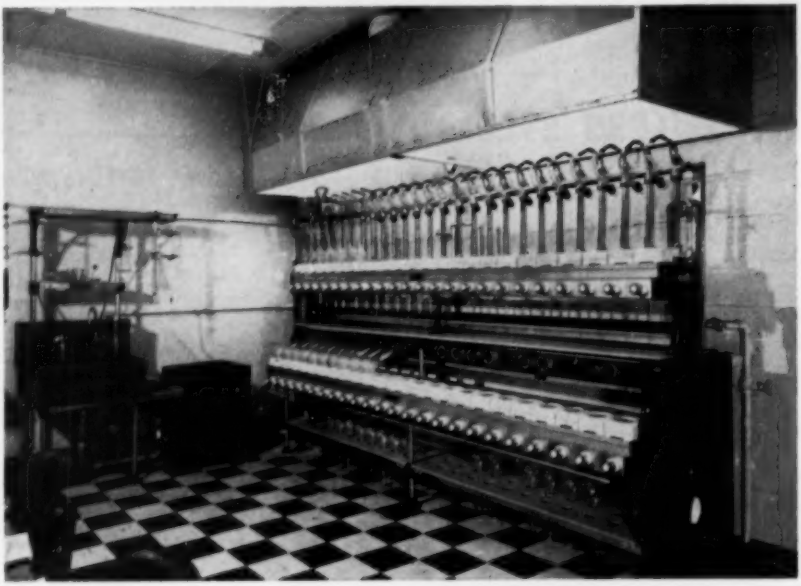

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CEREAL CHEMISTRY

VOL. 34 MAY, 1957 NO. 3

GRAPHICAL ANALYSIS OF THE BRABENDER VISCOSITY CURVES OF VARIOUS STARCHES[1]

EDWARD G. MAZURS, THOMAS JOHN SCHOCH, and FRANCIS E. KITE[2]

ABSTRACT

A system has been devised for the graphical analysis of the Brabender viscosity curves of various starch products. The complete cooking and cooling curves are first determined over a wide range of starch concentrations. The viscosities at five different critical points on these curves are then replotted against the log of the starch concentration. By this means, it is possible to compare thick-boiling and thin-boiling starches on the same scale, and to delineate the specific viscosity characteristics of various modified starch types.

The Brabender Amylograph has been extensively used in this laboratory to chart the changes in consistency which occur during the pasting, cooking, and cooling of aqueous starch systems. In particular, this instrument has been applied to the various modified and derivatized starches[3] and to the low-soluble gums and dextrins, as an indication of the behavior of these products in actual use. With each product, the complete cooking and cooling curves have been determined at five to eight different starch concentrations to cover the measuring range of the Brabender instrument. This has given an unwieldy mass of data which has become increasingly difficult to correlate and interpret. As a further complication, the thin-boiling starch products are necessarily run at much higher starch concentrations than the thick-boiling unmodified starches, and hence it has frequently been difficult to discern the properties of a particular starch product divorced from the influences of concentration. Some mode of classification has therefore been sought which would more readily delineate the important consistency characteristics without giving undue weight to starch concentration. This has been accom-

[1] Manuscript received August 7, 1956.
[2] George M. Moffett Research Laboratories, Corn Products Refining Company, Argo, Ill.
[3] In the nomenclature of the industry, the term "derivatized starches" refers to those products which have been chemically reacted to introduce substituent groups (e.g., ether, ester) into the starch molecule. The term "modified starch" is commonly applied to products which have been treated with hydrolyzing or oxidizing agents, usually to reduce the viscosity or to increase the solubility.

plished by a system of graphical analysis which transposes the Brabender data into a more readily comprehended form.

Methods and Materials

Brabender Procedure. The standard model Amylograph (marketed by the Brabender Corporation, Rochelle Park, N. J.) was used in these studies. It has been general practice in this laboratory to calibrate the Amylograph against National Bureau of Standards oils No. M and No. N. Five different instruments have been calibrated in this fashion over the range of 1 to 14 poises. The relationship between poises and Brabender units is almost linear, and the average deviation from the mean for the five instruments is $\pm$ 6%.

In the current procedure for determining the complete Brabender curve, an appropriate amount of starch (on dry basis, by prior moisture determination) is slurried in distilled water and made up to 500 ml. in a volumetric flask. The slurry is then transferred to the cup of the Brabender Amylograph and heated at a rate of 1.5°C. rise per minute. The temperature is observed by a short-stem thermometer mounted to dip directly into the starch paste, rather than by the slug in the Brabender thermoregulator. The starch paste is heated to 95°C., maintained at that temperature for one hour, then cooled at a rate of 1.5°C. per minute to 50°C. and held for an hour. With those starches having high pasting peaks, the recorder may go off the chart. In such cases, the rotating unit is held by a retaining peg until the paste thins. The cooking temperature of 95°C. was employed as the maximum safe operating temperature of the instrument; this permitted the development of optimal swelling of the starch granules. Lower pasting temperatures give completely different viscosity curves; for example, the cereal starches may show no viscosity peak when cooked at 90°C. A temperature of 50°C. was arbitrarily chosen for evaluation of set-back on cooling. Obviously, starch products are pasted and used under a wide variety of conditions, and no standard cycle of cooking and cooling can be devised which will be generally representative of their diverse applications. However, it is believed that the foregoing procedure serves to demonstrate the technologically important consistency characteristics of the various starches.

Graphical Analysis. If the Brabender curves at a variety of starch concentrations are replotted on rectangular coordinates and properly superimposed, they give a pattern of the type illustrated in Fig. 1 for unmodified corn starch. There are five successive points of significance on these curves:

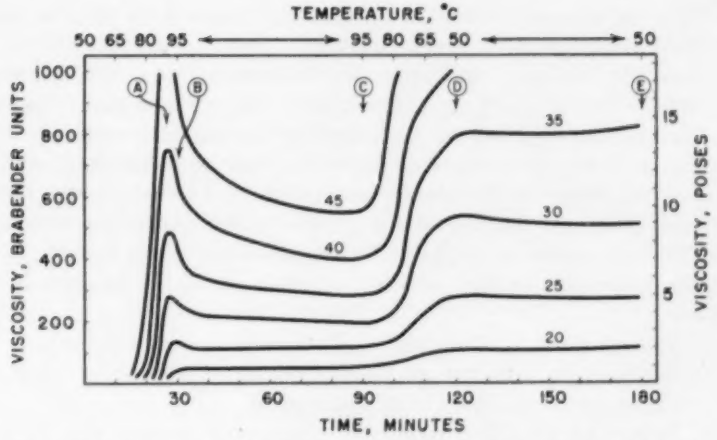

Fig. 1. Brabender curves of thick-boiling corn starch. Letter keys indicate viscosity reference points. Numerals indicate starch concentration, in g. per 500 ml.

A—Peak viscosity, irrespective of the temperature at which the pasting peak is attained. This is important to the user because in most cases he must cook *through* this stage in order to obtain a usable starch paste.

B—The viscosity when the paste reaches a temperature of 95°C. The relationship of this value to the peak viscosity reflects the ease of cooking the starch.

C—Viscosity after cooking for one hour in the Brabender at 95°C., which indicates the stability or breakdown of the paste during cooking.

D—The viscosity when the cooked paste is cooled to 50°C. in the Brabender, as a measure of the set-back produced by cooling.

E—The final viscosity after stirring in the Brabender for one hour at 50°C., to indicate the stability of the cooked paste in actual use.

If for each of the above significant points, the viscosity is plotted against starch concentration, a second set of curves is obtained for each starch. The resulting curves for the thick-boiling starches will be very steep, while those for the thin-boiling products will show a much more gradual rise. Obviously, this only accentuates the difference between thick and thin starches and does not reveal those inherent properties which are due to derivatization or other modification. However, with the majority of starch products, the viscosity range of the Brabender instrument accommodates an approximate

doubling of the starch concentration (viz., from 4 to 8% for unmodified corn starch, or from 20 to 40% for a white dextrin). To compare the viscosity-concentration relationship of a thick-boiling starch with that of a dextrin or a thin-boiling starch, it is advantageous to plot viscosity against the logarithm of the concentration. In this fashion, a direct comparison of the curves can be more easily made, since a doubling of the starch concentration represents the same distance along the horizontal log axis, whether for a high-viscosity thick-boiling starch or for a thin white dextrin.

The procedure in the present system of graphical analysis is as follows:

1. The complete cooking and cooling curves are determined at five to eight different starch concentrations, chosen to cover the range of the Brabender instrument.
2. From the resulting family of Brabender curves, the five significant points (listed as A to E above) are selected.
3. The viscosities at each of these points are then replotted against the log of the corresponding concentration, to give five curves which show the influence of concentration on the significant stages in the cooking and cooling cycle.

For convenience in plotting, a master chart may be prepared in which the vertical axis of 10 inches represents 1000 Brabender units. The horizontal axis is a two-cycle logarithmic scale, 20 inches per cycle in length, representing concentration (as g. of dry starch per 100 ml. of slurry). Obviously, only a small segment of this master chart will be used for any one starch. It is recommended that the curves for a particular starch be plotted in color on a sturdy transparent drafting material (e.g., "Stabilene," marketed by Keuffel and Esser), the peak viscosity being indicated by a solid black line, the initial and final 95°C. curves by dotted red and solid red curves, respectively, and the initial and final 50°C. curves by dotted blue and solid blue curves, respectively. Such a color code greatly facilitates recognition of the important viscosity characteristics of the starch. Also, by the use of a transparent base, the curves can be superimposed on one another for comparison, either by alignment at equal concentrations on the horizontal log axis or by direct comparison of curve form and shape without consideration of concentration.

These techniques have been applied to a wide variety of commercial and experimental products. For present purposes, ten different starch types have been selected to illustrate the diverse consistency characteristics which are encountered. The products are described in terms of industrial tests for Scott hot-paste viscosity, alkali fluidity,

and dextrin solubles; details of these procedures are given by Kerr (1). In the present publication, the curves in the various charts (Figs. 2 to 11) are identified as follows:

A. Dotted line representing the pasting peak. (This curve is absent with certain cross-bonded starches.)
B. Broken line for initial 95°C. viscosity.
C. Solid line for final 95°C. viscosity after one hour of cooking.
D. Broken line for initial 50°C. viscosity.
E. Solid line for final viscosity after 1 hour at 50°C.

Results and Discussion

The logarithmic charts of these ten starch products are presented in Figs. 2 to 11, inclusive. The salient features of these curves are described under the following headings.

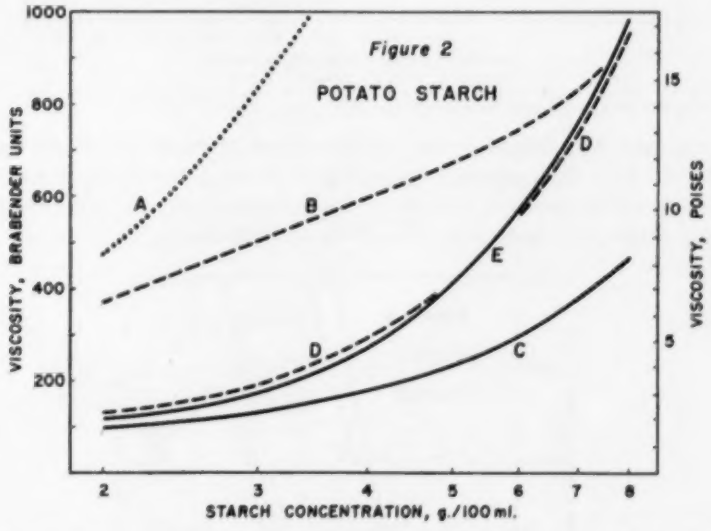

Fig. 2. Commercial unmodified potato starch ("Nasco" brand, Northern Aroostook Starch Co., Fort Kent, Maine), 3-g. Scott = 95 (i.e., seconds for 100 ml. delivery).

Concentration Range. Maine potato starch (Fig. 2) and cationic corn starch (Fig. 3) show a slow and progressive rise in viscosity with increasing concentration. In contrast, the viscosity curves of unmodified wheat starch (Fig. 4) ascend rapidly with increasing concentration. Thick-boiling corn starch (Fig. 5) and tapioca starch (Fig. 6) are intermediate in character. Those products with more gradually ascending viscosity curves are not as sensitive to variation in concen-

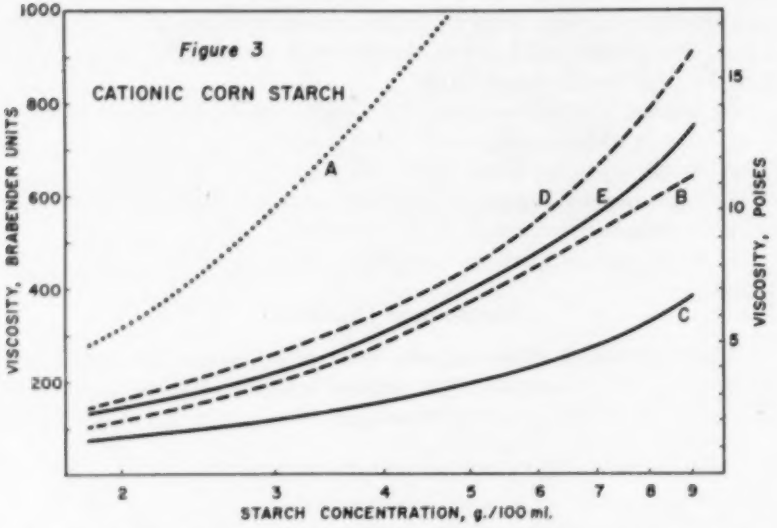

Fig. 3. Cationic corn starch, experimental thick-boiling positively-charged product similar to that described by Kerr (2), 2-g. Scott = 41.

tration and may be used over a wider range of starch solids. In contrast, the corn dextrin depicted in Fig. 7 shows a sudden and almost vertical rise in viscosity; such a product obviously should not be used at concentrations exceeding 45 to 50 g. per 100 ml.

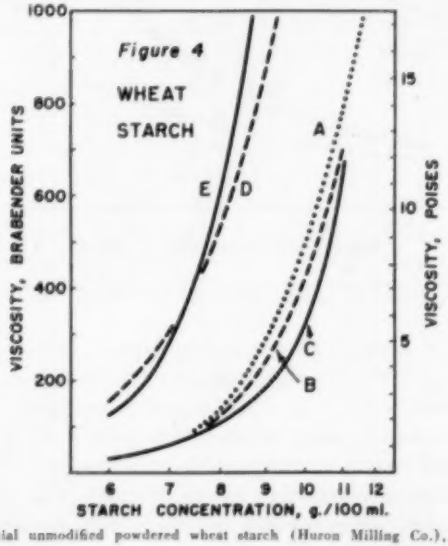

Fig. 4. Commercial unmodified powdered wheat starch (Huron Milling Co.), 15-g. Scott = 64.

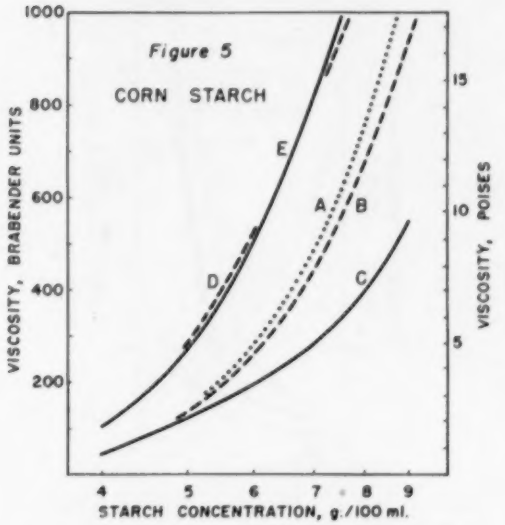

Fig. 5. Commercial unmodified corn starch, 12-g. Scott = 76.

Peak Viscosity. Potato starch exhibits a very high and sharp viscosity peak followed by pronounced thinning, as shown by the relative positions of Curves A and B in Fig. 2. Cationic corn starch and waxy sorghum starch (Fig. 8) give similar patterns. Tapioca shows

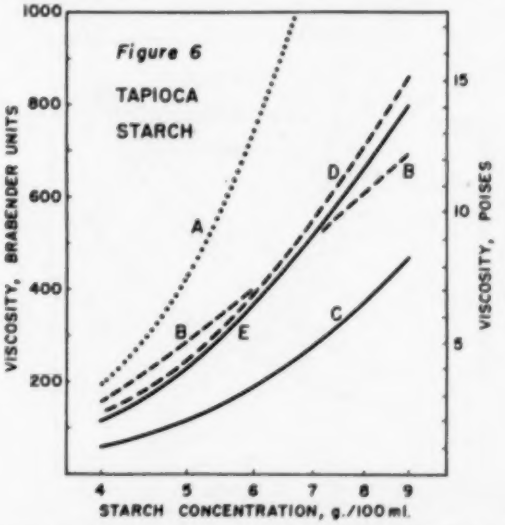

Fig. 6. Commercial unmodified tapioca starch ("Maris" brand, Stein Hall), 8-g. Scott = 69.

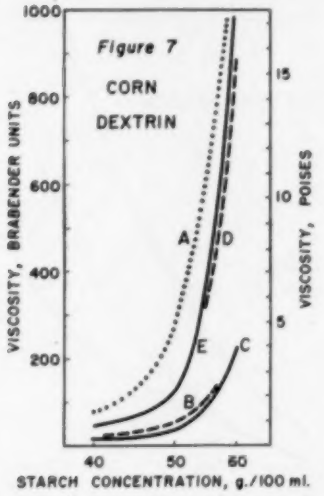

Fig. 7. Commercial corn white dextrin, 43% soluble in cold water.

a moderately high peak viscosity. This effect obviously reflects fragility of the swollen granules, which first swell and then break down under the continuous mechanical stirring of the Brabender. These starches therefore require careful cooking with good agitation, in order to pass through the viscosity peak and achieve a properly cooked paste. Unmodified corn starch shows almost no viscosity peak, i.e., the curve

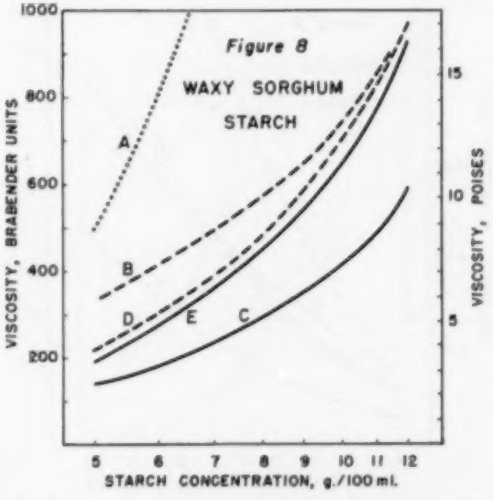

Fig. 8. Commercial unmodified waxy sorghum starch, 8-g. Scott = 106.

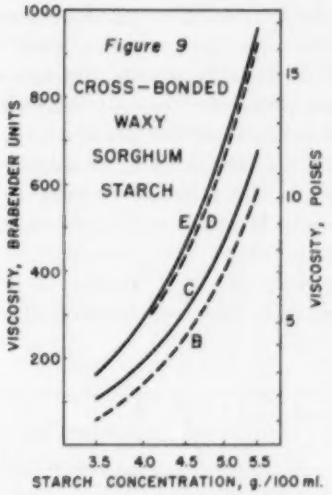

Fig. 9. Cross-bonded waxy sorghum starch, manufactured from regular waxy sorghum starch by chemical cross-bonding within the granule, 11-g. Scott = 82.

for initial 95°C. viscosity is very little lower than that for the peak viscosity. This behavior reflects the greater stability of the swollen corn starch granule against mechanical disintegration. Granular starches which have been chemically cross-bonded with such agents as formaldehyde, epichlorohydrin, or phosphate ester linkages show no peak viscosity at all. The cross-bonded waxy sorghum starch shown

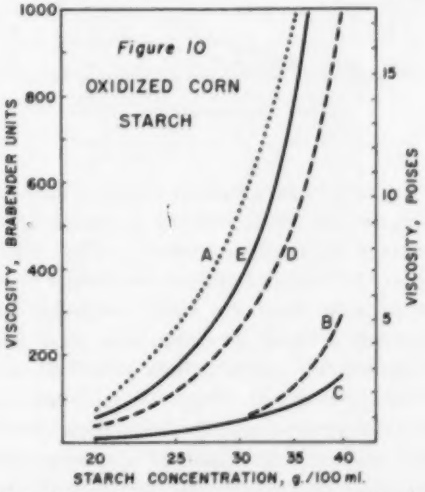

Fig. 10. Commercial hypochlorite-oxidized corn starch ("Hercules" brand), 100-g. Scott = 56.

in Fig. 9 is a typical example. This product shows major differences in paste character from the parent waxy sorghum starch (compare Fig. 8). The presence of internal cross-linkages retards and restricts granule swelling, and hence the viscosity increases perceptibly during cooking. These cross-bonded starches are not fragmented or solubilized by prolonged cooking, i.e., the amount of soluble starch leached from the swollen granule into the aqueous substrate is much less than with unmodified corn starch. If any retrogradation or gelation does occur on cooling, it must therefore take place within the swollen granule and not in the aqueous substrate. Hence each swollen granule remains physically separate, and consequently the set-back on cooling is minimal.

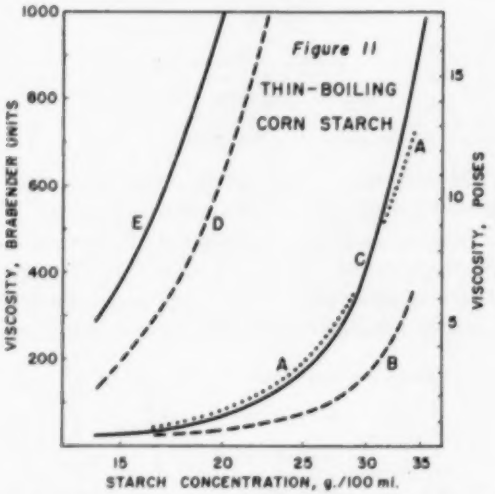

Fig. 11. Commercial 80-fluidity acid-modified corn starch, 75-g. Scott = 55.

Hot Paste Viscosity. Most granular starches thin down during the 1-hour cooking period at 95°C., owing to progressive fragmentation and solubilization of the swollen granules. This effect is particularly evident in the great difference between the initial and the final 95°C. curves for potato starch. Tapioca, waxy sorghum, and cationic corn starch likewise show marked thinning, but to a lesser degree. Unmodified corn and wheat starches show relatively little thinning on cooking. Oxidized corn starch (Fig. 10) and corn dextrin (Fig. 7) show almost no change on cooking, since these products dissolve in hot water to give molecularly dispersed solutions, rather than pastes of swollen granules. As previously mentioned, the cross-bonded

starches show increased viscosity on cooking. The only other instance of thickening during cooking was with an 80-fluidity thin-boiling corn starch (Fig. 11). This latter product is likewise the only case as yet encountered where the graphical log chart does not present a complete and accurate description of paste behavior. The actual Brabender curves at higher concentrations (Fig. 12) show that the set-back occurs during the first 15 minutes at 95°C., followed by appreciable thinning during the remainder of the cooking period. As a plausible

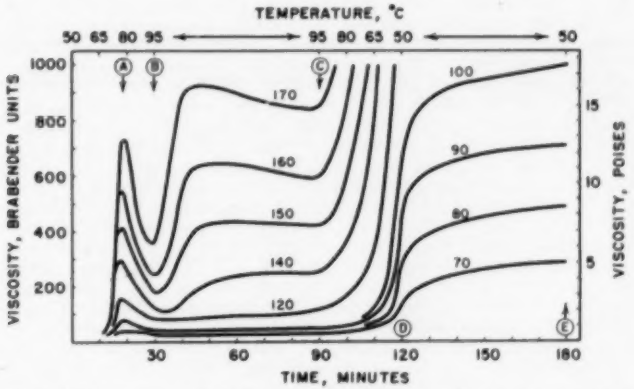

Fig. 12. Brabender curves of 80-fluidity acid-modified corn starch. Letter keys indicate viscosity reference points. Numerals indicate starch concentration, in g. per 500 ml.

explanation, the granules may first swell and then dissolve to give a molecularly dispersed solution. At high concentrations, the latter is unstable and tends to set up to a gel even while holding at 95°C. Continued mechanical agitation thereafter breaks down this gel structure, with consequent thinning of the hot paste.

Set-Back on Cooling. The extent of increase in viscosity on cooling to 50°C. reflects the retrogradation tendency of the starch product. Of these ten starches, cross-bonded waxy sorghum shows minimum set-back, while the 80-fluidity corn starch shows maximum increase in viscosity. While it is not shown here, there is a progressively widening divergence between the 95°C. and the 50°C. curves as corn starch is acid-modified through the various commercial thin-boiling levels to the 80-fluidity product. This may be attributed to a sort of "mass action" effect—i.e., the thinner the starch, the higher are the concentrations employed, and consequently the greater is the opportunity for association of the molecules. A similar progressive divergence between the hot and cold paste viscosities is observed with a series of acid-modified hydroxyalkyl starches of various fluidities. Also, at any

single fluidity level, there is no significant difference between the viscosity patterns of hydroxyalkyl starches of 0.04 and 0.1 degree of substitution. This increase in derivatization apparently does not alter the viscosity characteristics, though other advantages may be realized (increase in paste clarity, higher solubility of the dried film). Set-back frequently continues while holding at 50°C., as exemplified by the 80-fluidity corn starch. In contrast, waxy sorghum and cationic corn starch show a slight thinning during the 1-hour period at 50°C.

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FACTORS AFFECTING COLOR STABILITY OF STORED SUGAR COOKIES[1]

THOMAS GRIFFITH, JOHN A. JOHNSON, and JACK I. NORTHAM[2]

ABSTRACT

The effect of several atmospheric factors on color stability during storage of dextrose and sucrose sugar cookies was studied. The cookies containing 5% dextrose were initially brown in color due to the Maillard reaction, whereas the sucrose cookies essentially lacked brown pigments.

Storage of cookies containing dextrose in a high relative humidity 42 days caused the darkness of the color to decrease, whereas color changes in the sucrose cookies were slight. The fading of the brown color is closely related to the relative humidity.

Changes in the color also depended on the gaseous atmosphere in which the sugar cookies were stored. At 10% relative humidity (R.H.) the effect of gaseous atmosphere was slight, but at 34% R.H., decrease in darkness of the dextrose cookies was greatest when the cookies were stored in carbon dioxide. Significantly less decrease in darkness was observed when the cookies were stored in oxygen and nitrogen.

Changes in the color of the cookies were related to the temperature of storage. Cookies stored at 10% R.H. were not affected significantly by storage temperatures. The sucrose cookies stored in 34% R.H. at 25° and 38°C. exhibited no significant decrease in darkness, while at 70°C. the cookies became increasingly brown as the time of storage was extended. A significant effect of temperature was exhibited on dextrose cookies stored in 34% R.H. Storage at 25° and 38°C. resulted in a loss of color, while storage at 70°C. resulted in an initial loss of color followed by an increase in darkness of color. The development of the brown color in the "dry state" appears to occur during storage of cookies and depends primarily on humidity and temperature.

A brown color may be produced in sugar cookies by the addition of a small percentage of reducing sugar to the formula. The formation of brown pigments has been shown to be due primarily to the Maillard type browning reaction, which occurs during the baking process (4). While knowledge of this reaction is incomplete, it is recognized that under certain storage conditions, browning of some products such as dry milk (5) and eggs (6) may occur. In contrast, it has been observed that under some conditions sugar cookies may decrease in darkness of color with storage time (9).

Retention of color in bakery products is an important economic factor. This is true, particularly, of products such as cookies that may have an extended "shelf life." It is generally known that color stability of cookies is better when they are stored in a vacuum pack

[1] Manuscript received August 27, 1956. Cooperative investigation between the Department of Flour and Feed Milling Industries and Statistical Laboratory, Kansas Agricultural Experiment Station, Manhattan. Contribution No. 251, Department of Flour and Feed Milling Industries, and No. 10, the Statistical Laboratory, Kansas Agricultural Experiment Station, Manhattan. Presented at 40th annual meeting, St. Louis, Missouri, May 1955.

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than when stored in contact with the normal atmosphere. Although the evidence regarding these observations is limited, it suggests that atmospheric conditions may affect the stability of the brown melanoidin pigments during storage. It, therefore, appeared of value to investigate the effects of certain atmospheric conditions, including humidity, gaseous atmosphere, and temperature, on the color stability of sugar cookies.

Materials and Methods

Two types of cookies were baked for storage tests. They were prepared according to the following formulas (9):

Ingredient	*Percentage*[a]	*Quantity*
	%	g.
Flour	100.0	600.0
Sugar[b]	60.0	360.0
Shortening[c]	30.0	180.0
Sodium bicarbonate	1.75	10.5
Salt	1.0	6.0
Water	27.0	162.0

[a] Based on flour weight.
[b] Type I cookie was prepared using 60% sucrose. Type II cookie was prepared using 5% dextrose and 55% sucrose.
[c] Primex B & C shortening, Procter & Gamble Co., Cincinnati, Ohio.

The sugar, shortening, sodium bicarbonate, and salt were creamed at medium speed using the Hobart N-50 mixer, after which the water was added and the batter mixed until homogeneous. The flour and batter then were incorporated into a dough. The cookie doughs were rolled to a thickness of 6 mm. and cut into pieces 59 mm. in diameter. Baking was at 425°F. for 10 minutes.

Cookies of each type were selected from larger batches of cookies on the basis of uniformity of color. The 60 cookies of each type were selected for each separate experiment, involving the effects of humidity, atmospheric gases, and temperature. For each experiment and cookie type, the 60 cookies were randomly assigned (3). Ten cookies were placed in each of six 1-gal. jars equipped with tight-fitting lids. A statistical analysis, for each experiment and each cookie type, showed that the mean initial reflectance did not vary significantly among the six jars. Ten cookies, supported over appropriate saturated-salt solutions by plastic disks, were separated in the jars by small pieces of aluminum foil. The cookies were stored in a dark incubator at 38°C., unless the effect of temperature was being studied.

External color was determined with a Photovolt Reflectometer, Model 610, equipped with a green tristimulus filter (9). Three color-reflectance readings were taken of each cookie at regular intervals for

the duration of the experiment. The experiments involving the effect of atmospheric gases and temperature were stopped after 28 days of storage; the experiment involving the effect of humidity was extended to 42 days. The data were analyzed statistically by methods of Box (2).

The relative humidities were maintained by use of various saturated-salt solutions (1) placed in the bottom of the glass jar. Cookies stored at 0% R.H. were placed in a desiccator containing calcium chloride.

In experiments involving atmospheric gases and temperature, two humidity levels were used. A 34% or 10% R.H. was maintained with saturated solutions of potassium carbonate and lithium chloride.

Storage of the cookies in three gases, carbon dioxide, oxygen, and nitrogen, was studied. The jars containing the cookies were first evacuated for 5 minutes, then flushed for 5 minutes with a given gas under 5 lb. pressure. The gases were replenished every fourth day after color measurements were taken.

The cookies used to study the effect of temperature were stored at 25°, 38°, and 70°C.

Results and Discussion

Effect of Humidity. The effects of relative humidity and storage time on color stability of sugar cookies are presented in Fig. 1. A large decrease in darkness of color occurred with the dextrose cookies

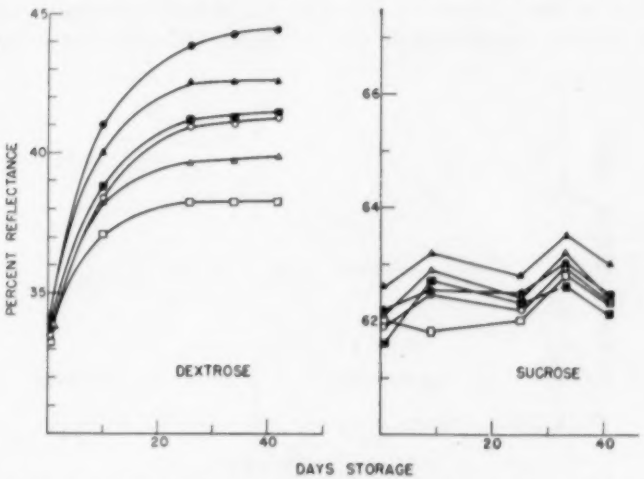

Fig. 1. Effect of relative humidity and storage time on the percent reflectance of sugar cookies stored in air. Solid circle, 67.7% R. H.; solid triangle, 43.4% R. H.; open circle, 31.9% R. H.; solid square, 20.4% R. H.; open triangle, 11.1% R. H.; open square, 0% R. H.

for all humidity levels during the first 10 days of storage. The extent of color fading was greatest at R.H. 67.7%. With the exception of the groups stored at 20.4 and 31.9% R.H., the extent of color fading was closely related to the relative humidity in which the cookies were stored. A similar loss of color darkness for each humidity level was noted at 26 days of storage. The change in color after 26 days was negligible.

Statistical analysis showed that the mean daily rates for decrease in darkness for the full 42 days of storage were significantly different from one humidity group to the next. Also, the shapes of the curves, on the whole, were significantly different with the exception of the 20.4 and 31.9% humidity groups. No attempt was made to determine the fate of the colored compounds; these may have been drawn into the center of the cookie or they may have undergone some type of decomposition.

The sucrose cookies did not vary in the mean daily rates of color-fading from one humidity level to another. Since these cookies were initially lacking in brown color, it was to be expected that humidity would have no significant effect. The irregularity of the percent reflectance during the 40-day period of observation cannot be explained readily.

Effect of Gaseous Atmosphere. The effects of gaseous atmospheres, humidity, and storage time on color changes are shown in Fig. 2. The most striking feature of these data is the effect of the various gases on the color change in the dextrose cookies compared with that of the sucrose cookies stored in 34% R.H. Although not illustrated

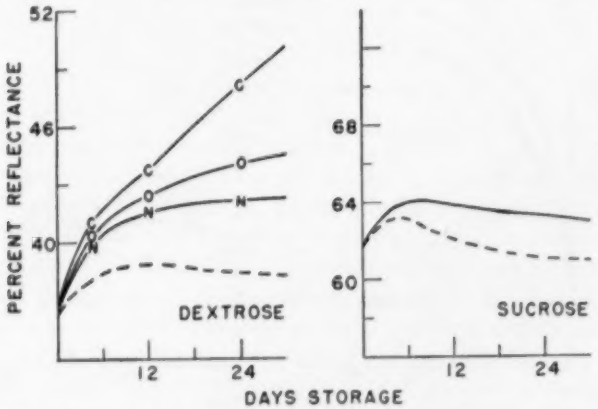

Fig. 2. Effect of gaseous atmosphere, relative humidity, and storage time on percent reflectance of sugar cookies, stored at 38°C. Solid line, 34% R. H.; broken line, 10% R. H. C, carbon dioxide; O, oxygen; N, nitrogen.

for the sucrose cookie data, statistical analysis showed that the color changes of the sucrose cookies stored in an atmosphere of oxygen and carbon dioxide and in 34% R.H. were significantly different from the cookies stored in nitrogen. The sucrose cookies, stored in various gases and in 10% R.H. were significantly different as a group at the end of the storage period. In Fig. 2 separate graphs are not drawn for the various gases at 34 and 10% R.H. because the differences, while statistically significant at the end of 28 days, were of the order of 1% and were not deemed to be of great practical significance. The sucrose cookies stored in 34% R.H. were lighter in color than those stored in 10% R.H.

The type II (dextrose) cookies stored in various gases and in 10% R.H. were not significantly different, but when they were stored in 34% R.H. the effects of the gaseous atmospheres were marked. Greatest loss of brown color occurred in dextrose cookies stored in carbon dioxide. Cookies stored in oxygen retained significantly more color than those stored in carbon dioxide, but showed somewhat greater loss of color than those stored in nitrogen.

Effect of Temperature. The effect of temperature on color change of type I and type II cookies stored in two different humidities is shown in Fig. 3. In either type of cookie, stored at 10% R.H., the temperature did not have a significant effect on color. Considerable changes in color were caused by temperature variation when the cookies were stored at 34% R.H. In the instance of the sucrose cookies stored at 34% R.H., results were essentially the same for storage temperatures of 25° and 38°C. This is illustrated in Fig. 3 by a line designated as 4 and this line represents a composite of the data

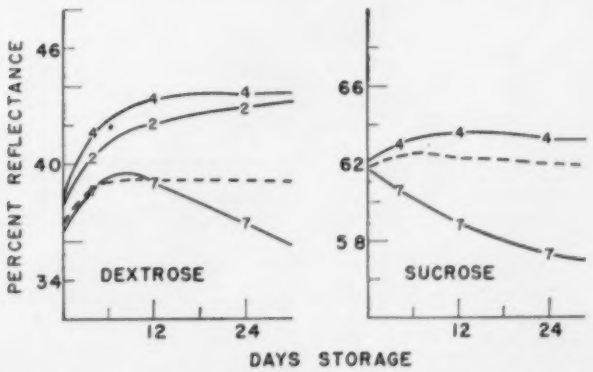

Fig. 3. Effect of temperature, relative humidity, and storage time on percent reflectance of sugar cookies, stored in air. Solid line, 34% R. H.; broken line, 10% R. H. The numeral 2 indicates 25°C.; 4 indicates 38°C.; and 7 indicates 70°C.

obtained at 25° and 38°C. At 70°C., browning of the cookies occurred. The interaction between temperature and humidity was significant.

In the instance of the dextrose cookies stored at 34% R.H., storage at 25° and 38°C. caused a significant decrease in darkness of color during the initial periods of storage, after which loss was much less. In contrast to this, cookies stored at 70°C. showed loss of darkness of color up to the 8th day and then became increasingly brown. This suggested that the browning-reaction products were being formed at a faster rate than they were being destroyed. The conditions are similar to those described for the browning reaction in the "dry state" (7, 8).

General Discussion

Several experiments serve to point out the effect of various atmospheric conditions on the stability of color of sugar cookies. It is significant that when a small percentage of sucrose is replaced with dextrose, a Maillard browning reaction occurs. Since sucrose cookies are lacking in brown color, they can be expected to be more stable than dextrose cookies under all conditions of storage.

The atmospheric conditions which cause a decrease in darkness of the cookie are: 1) storage in high relative humidity and 2) storage in a carbon dioxide gas. If the temperature is increased to 70°C., an increase in darkness occurs in both types of cookies when stored at high relative humidities. These conditions agree with those that promote the browning reaction in the "dry state" (7, 8).

Stability of the brown color during storage is enhanced by packing of the sugar cookies in low relative humidity and in an inert gas and by storage at lower temperatures.

Acknowledgment

Acknowledgment is given to Corn Products Sales, Inc., for financial assistance in making this study possible.

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RELATION OF THE BROWNING REACTION TO STORAGE STABILITY OF SUGAR COOKIES[1]

Thomas Griffith and John A. Johnson[2]

ABSTRACT

The effect of products of the browning reaction on fat stability in stored sugar cookies was studied. These compounds were characterized by various methods.

It was demonstrated that the addition of 5% dextrose to sugar cookies produced a marked browning in the cookies and these cookies exhibited greater stability to oxidative rancidity than cookies in which no browning occurred.

Lyophilized water extracts of the browned sugar cookies and a synthetic glycine-dextrose browning reaction mixture exhibited antioxidant properties. Extracts of cookies in which little or no browning occurred demonstrated very limited antioxidant properties. Antioxidant properties were associated with the presence of reductones formed during the browning reaction.

Specific tests demonstrated a relationship between the concentration of reductones in sugar cookies and model systems and antioxidant properties.

Literature concerning the Maillard-type browning reaction is voluminous, as indicated by two recent reviews (8, 16). However, very little of the literature deals with the browning of bakery products. It has been established by Bertram (4) and Haney (12) that Maillard-type browning is the main source of the brown pigments formed during the baking of bread. Haney (12) demonstrated a similar type of browning in sugar cookies.

The browning reaction is very complex and many compounds are produced during this reaction (7, 13). The primary condensation product of the sugar and amine is a N-substituted glycosyl amine (19, 21); however, this compound does not account for all the properties exhibited by browning-reaction systems. Several investigators (3, 10, 13, 22) postulated that the Amadori rearrangement of N-substituted gly-

[1] Manuscript received August 27, 1956. Contribution No. 252, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. This research is a portion of a thesis presented by Thomas Griffith as partial fulfillment of the requirements for the Degree of Master of Science in Flour and Feed Milling Industries at Kansas State College. Presented at the 40th annual meeting, St. Louis, Missouri, May 1955.

[2] Respectively, Research fellow and Professor, Department of Flour and Feed Milling Industries, Kansas State College, Manhattan.

cosyl amines (23) would explain some of their experimental results. Recently, Hodge and Rist (17, 18) have demonstrated the Amadori rearrangement with amines and dextrose. From the Amadori rearrangement product, a 1-amino-1-desoxy-2-ketose, they have demonstrated several reactions. In one, reductones were formed.

It has been observed in this laboratory that sugar cookies prepared with 60% sucrose became rancid in a shorter period than did sugar cookies prepared with 55% sucrose and 5% dextrose. At the time no evidence was available that would indicate the nature of this inhibition; however, since reducing sugar participates in the browning reaction, it is possible that some product or products formed during the course of this reaction may be responsible for the increased stability of the fat in the sugar cookies.

The purpose of this investigation was to establish the nature of the substances that inhibit the development of rancidity and their relation to the browning reaction.

Materials and Methods

Storage Studies. Sugar cookies were prepared and stored as previously reported (11). The effect of moisture and storage time on the development of a rancid condition was studied by storing the cookies at 100°F. in various relative humidities produced by saturated salt solutions (1).

The effect of gaseous atmospheres and storage time on the development of rancidity in sugar cookies was studied by using oxygen, nitrogen, carbon dioxide, and air atmospheres. The jars containing the cookies were recharged with the respective gases after each sampling.

Several analyses were performed at each sampling. The presence or absence of an organoleptically rancid condition was determined by three persons noting the odor of the sugar cookies immediately upon opening the jar. The pH of the sugar cookies was determined by using a line-operated Beckman pH meter, Model H-2 (2). The peroxide number of the fat contained in the cookies was determined using three samples of cookies which were ground in a Hobart grinder and extracted with 20 ml. of acetic acid or citric acid reagent (14). After filtering, the peroxide content was determined by the method of Hartman and White (14).

Characterization of the Reducing Compounds. For these studies sugar cookies were prepared as described previously (11), but with lard containing no antioxidant additive.

Water extracts of these cookies were prepared by mixing 200 g. of sugar cookies with 500 ml. water for 2 minutes in a Waring Blend-

or followed by a 15-hour steeping period. The fermentable sugars in the filtrate were removed by yeast fermentation (*Saccharomyces cerevisiae*), and clear solution was obtained by centrifugation after 24 hours of fermentation. The extracted material was lyophilized and stored at −10°F. While it is possible that some reducing substances may have been formed through fermentation, the procedure provided extracts that were comparable for the two types of cookies being investigated. Bread-crust extract was obtained in a similar manner.

Synthetic browning-reaction products were produced by heating a 0.2 *M* glycine and a 0.5 *M* dextrose solution at 85°C. for 24 hours. The solution was dark brown but no precipitate was present. The final pH of the solution before lyophilization was 4.0.

A modified Swift keeping-quality test was used to determine the presence and relative concentration of fat antioxidant substances in the lyophilized extracts. Twenty milliliters of lard[3] containing no added antioxidants were placed in a 20 by 150-mm. test tube with a small quantity of the browning-reaction products and heated in an oil bath at 121°C. Oxygen was passed at a constant rate through the lard by means of a capillary tube. Measurements of peroxide concentration and organoleptic tests to confirm the presence or absence of rancidity were performed periodically.

Quantitative determinations of reducing groups were made by the Chapman-McFarlane ferricyanide test (6) as modified by Hlynka and Bass (15). The 2,6-dichlorophenolindophenol was prepared and purified by the method described by Bessey and King (5). The reducing power of the lyophilized extracts was determined in an alkaline solution (18). The oxidation of the enediol structure by *o*-dinitrobenzene was performed by the method of Fearon and Kawerau (9).

The reaction of iodine with the browning-reaction products was carried out in an acid medium. The lyophilized extracts (250 mg.) in 25 ml. of 10% acetic acid were titrated at room temperature with a standard iodine solution. Two milliliters of 1% starch solution were added as an indicator, and titrations continued until the blue color was stable for 1 minute.

Results and Discussion

Two types of sugar cookies were baked. The sucrose cookies, containing 60% sucrose, did not brown noticeably. Since the formula did not include milk and eggs, which contain reducing sugars, the only reducing sugar available was the small quantity present in the flour

[3] Obtained through the courtesy of Mr. Benjamin Lawrence, Procter and Gamble Co., Cincinnati, Ohio.

(20). The dextrose cookies, containing 55% sucrose and 5% dextrose, browned markedly.

Storage Studies. Cookies were stored under various relative humidities to study the effect of moisture content on their storage stability. The results shown in Tables I and II indicate that the relative humidity had no apparent effect on hydrogen-ion concentration in sugar cookies, even after 56 days of storage. Storage at high humidity caused the cookies to be more moist, as might be expected. The cookies containing dextrose faded considerably when stored at high humidities, confirming work reported previously (11). The color of the cookies containing sucrose did not change. The cookies containing dextrose tended to have slightly lower pH values than the sucrose cookies. These differences fail to account for the difference in the storage stability of the two groups.

The effect of relative humidity (R.H.) and storage time on the odor of sugar cookies is presented in Table III. A definite relationship be-

TABLE I

Effect of Moisture Content and Storage Time on the Hydrogen-Ion Concentration of Sucrose Cookies

Storage Time	Humidity (Percent)					
	11.1	20.4	31.9	41.1	50.0	67.7
	Cookie Moisture (Percent)					
	3.40	4.40	5.15	5.10	5.40	6.15
Days	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0	9.4	9.6	9.6	9.6	9.6	9.4
8	9.7	9.7	9.7	9.7	9.8	9.7
16	9.8	9.8	9.8	9.8	9.7	9.7
24	9.8	10.0	9.9	9.9	9.8	9.7
32	10.0	9.9	9.8	9.9	9.8	9.7
40	9.8	9.8	9.7	9.7	9.6	9.6
56	9.5	9.5	9.6	9.5	9.5	9.3

TABLE II

Effect of Moisture Content and Storage Time on the Hydrogen-Ion Concentration of Dextrose Cookies

Storage Time	Humidity (Percent)					
	11.1	20.4	31.9	41.1	50.0	67.7
	Cookie Moisture (Percent)					
	3.65	4.30	5.25	5.45	5.65	6.75
Days	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0	8.3	8.3	8.4	8.3	8.2	8.2
8	8.4	8.4	8.7	8.3	8.6	8.7
16	8.8	8.6	8.6	8.6	8.8	9.1
24	8.8	8.6	9.0	8.9	8.8	9.1
32	8.4	8.9	8.6	8.7	8.4	8.2
40	8.0	8.5	8.1	8.1	8.2	8.2
56	7.8	8.0	8.2	8.0	7.9	7.7

TABLE III

EFFECT OF MOISTURE CONTENT AND STORAGE TIME ON ODOR OF SUGAR COOKIES

Relative Humidity	Moisture Content		Time to a Rancid Condition	
	Sucrose	Dextrose	Sucrose	Dextrose
%	%	%	*days*	*days*
11.1	3.40	3.65	64	96
20.4	4.40	4.30	56	72
31.9	5.05	5.25	44	56
41.1	5.20	5.45	40	56
50.0	5.40	5.65	36	52
67.7	6.15	6.75	24	40

tween humidity and time required for a rancid condition to develop was established for both types of cookies. At 67.7% R.H., organoleptic rancidity was detected after 24 and 40 days in the sucrose and dextrose cookies, respectively. At 11.1% R.H., rancidity was detected only after 64 and 96 days for the sucrose and dextrose cookies, respectively. Thus, storage at higher moisture levels accelerated the onset of an organoleptically rancid condition in the sugar cookies. The addition of 5% dextrose to the sugar cookies retarded the onset of rancidity in all cases. These results confirmed preliminary results carried out in this laboratory (24), and suggest that some product or products formed during the browning reaction inhibit oxidative rancidity in sugar cookies. Peroxide values were determined on the cookie fats. These data were erratic and failed to correlate with the organoleptic tests.

Cookies were stored under inert gases, air, and oxygen to determine the role of atmospheric oxygen in the formation of a rancid condition. The determination of peroxide numbers was used to follow the development of fat oxidation in the sugar cookies. As in the instance of the effect of humidity, these data also were erratic and no definite trends could be established. Organoleptic testing (Table IV) showed that the type of gaseous atmosphere had no effect on the development of organoleptic rancidity. It is recognized, however, that the minute quantities of oxygen dissolved or entrained in the cookies may have been sufficient to produce a rancid condition, independently of the gaseous atmosphere in which the cookies were stored. The data clearly demonstrated the important role of the browning reaction in retarding development of organoleptic rancidity. The addition of a small amount of dextrose increased the shelf life of the cookies approximately 70%.

Characterization of the Reducing Compounds. It was established in the storage studies that the addition of dextrose to sugar cookies increased the stability of the fat to oxidative rancidity. It was be-

TABLE IV

EFFECT OF GASEOUS ATMOSPHERE AND STORAGE TIME ON ODOR OF SUGAR COOKIES

ATMOSPHERE	TIME TO A RANCID CONDITION	
	Sucrose Cookie	Dextrose Cookie
	days	*days*
Air	60	100
Oxygen	60	100
Carbon dioxide	60	108
Nitrogen	60	108

lieved that this desired characteristic was due to the products of the Maillard-type browning reaction. In the course of a sugar-amine reaction Hodge and Rist (18) isolated a compound characterized as a reductone. These reductones contain in particular an enediol structure which can be readily oxidized or reduced. In view of the relationship between the systems under investigation and that of Hodge and Rist (18), studies were made to test for the presence and concentration of the reductones and their relation to fat stability in an isolated system.

The results of the application of the Swift keeping-quality test for determination of the effects of cookie extracts on lard are in Fig. 1.

The samples were considered organoleptically rancid at a peroxide number of 8.

The lyophilized water extract of the sucrose cookies exhibited weak fat antioxidant properties. The addition of 0.2 and 1.0% of this material caused the lard to exhibit increasing stability, although the change was small. A similar lyophilized extract of the dextrose cookie demonstrated considerably greater antioxidant properties. The increase in fat stability was shown to be related to the amount of cookie extract added to the lard.

Inhibition of the development of rancidity in lard by synthetic browning-reaction products and butylated hydroxy anisole is shown in Fig. 2. The stability of lard was increased considerably by the addition of 0.01% synthetic browning-reaction products. The addition of 0.1 and 0.2% of this material exhibited greater antioxidant properties. There was an increase of approximately 40% in the stability when 0.2% synthetic browning-reaction products were added to this lard. Butylated hydroxy anisole and the browning-reaction products gave similar curves. However, 0.005% of butylated hydroxy anisole exhibited greater fat antioxidant properties than 0.2% of the synthetic browning-reaction products.

The lack of effects of dextrose and bread-crust extract on fat stability are shown in Table V. The dextrose exhibited no appreciable effect on the fat stability. Similarly, bread-crust extract exhibited no

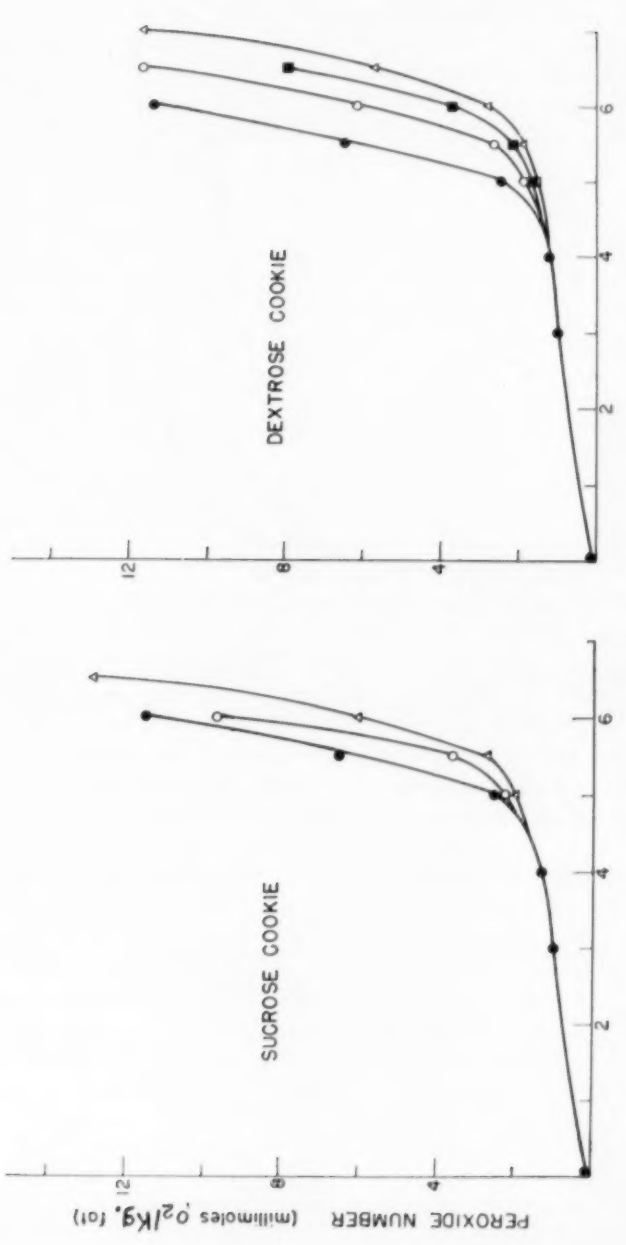

Fig. 1. Effect of cookie extracts on the stability of lard at 121°C. Solid circles, standard; open circles, 0.2% extract; solid squares, 0.6% extract; open triangles, 1.0% extract.

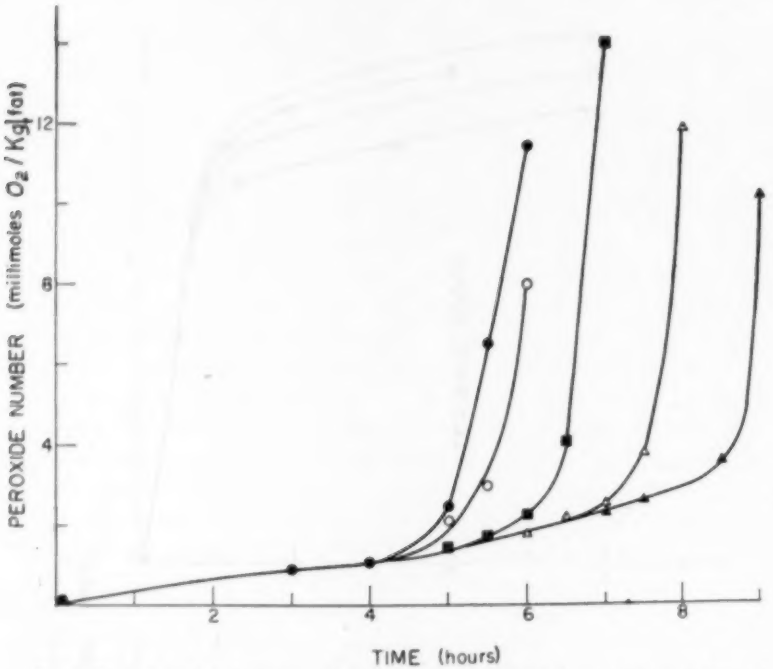

Fig. 2. Effect of synthetic browning-reaction products and butylated hydroxy anisole on stability of lard at 121°C. Solid circles, standard; open circles, 0.01% browning reaction products (BRP); solid squares, 0.1% BRP; open triangles, 0.2% BRP; and solid triangles, 0.005% butylated hydroxy anisole.

effect on the fat stability. These later results do not appear tenable at present, in view of the fact that nonfermentable reducing compounds should also be present in bread crust as a result of the browning reaction.

The results of the Chapman-McFarlane test (Table VI) showed the

TABLE V

EFFECT OF ANHYDROUS DEXTROSE AND BREAD-CRUST EXTRACTS ON LARD STABILITY

TIME	PEROXIDE NUMBERS		
	Standard Lard	Anhydrous Dextrose, 0.2%	Bread Crust Extract, 0.5%
Hours	*mM O₂*	*mM O₂*	*mM O₂*
0.0	. . .	. . .	. . .
3.0	0.88	0.90	. . .
3.5	. . .	. . .	0.95
4.0	1.00	1.30	. . .
4.5	. . .	. . .	1.91
5.0	2.52	2.17	. . .
5.5	6.45	4.08	4.42
6.0	11.83	16.90	13.51

presence of reducing compounds. It was of interest that the extract from sucrose cookies had but a small amount of reducing groups, whereas the dextrose cookies and the dextrose-glycine reaction mixture showed an appreciable amount. It was evident also that there was a relationship between the concentration of reducing groups and the stability to oxidative rancidity imparted to lard (Figs. 1 and 2).

TABLE VI

REDUCING PROPERTIES OF SYNTHETIC BROWNING-REACTION PRODUCTS AND SUGAR-COOKIE EXTRACTS

REDUCING TEST	SYNTHETIC BROWNING-REACTION PRODUCTS	DEXTROSE-COOKIE EXTRACT	SUCROSE-COOKIE EXTRACT
Chapman-McFarlane ferricyanide (6) (moles reducing groups $\times 10^{-5}$/g.)	72	26.4	6.3
o-Dinitrobenzene (9)	Violet	Violet	No color
2,6-Dichlorophenolindophenol (5, 18)	Rapid decolorization	Rapid decolorization	Very slow decolorization
Reaction with iodine (moles $I_2 \times 10^{-5}$/g.)	7.04	3.71	2.24

The small amount of reducing groups found in sucrose cookies was correlated with the lack of stability to storage of these cookies. The slight inhibition of rancidity in the sucrose cookies may be due to a small amount of browning that resulted from the presence of traces of reducing sugar in the flour. Natural antioxidants in the flour also may account for the limited stability of the sucrose cookies. The dextrose cookie extract contained about four times more reducing groups than did the sucrose cookie extract. This increase in reducing groups was due to the products of the browning reaction.

Further characterization of the reducing properties of the dextrose cookie extract was made by use of 2,6-dichlorophenolindophenol and *o*-dinitrobenzene (Table VI). It was shown that synthetic browning-reaction products and dextrose cookie extract reduced 2,6-dichlorophenolindophenol rapidly, whereas the reducing groups in sucrose cookie extract reacted very slowly. Evidence for the presence of the enediol structure in the browning-reaction products was obtained by means of *o*-dinitrobenzene. Synthetic browning-reaction products and dextrose cookie extracts gave positive tests. The test was negative for the sucrose cookie extract. This indicated that reductonelike compounds were formed during the browning reaction in the dextrose cookie, whereas no such compounds were detected in the sucrose cookies.

The data in Table VI demonstrate that some products produced during the browning reaction had the ability to reduce, add, or react with the iodine. The course of the reaction is not known. The sucrose cookie extract reacted with an appreciable quantity of iodine, even though very little browning occurred. The dextrose cookie extract reacted with a considerably larger quantity of iodine, indicating that the browning-reaction products formed in sugar cookies do react with iodine in some manner. The quantity of iodine which reacted appears to be related to the concentration of reducing groups. Since one mole of reductone will react with two equivalents of iodine, these results support the postulate that reductones are formed during the browning reaction.

These data indicate that reducing groups were produced in a dextrose cookie during the formation of the brown melanoidin pigments. The reducing groups appear to be similar in reaction to those produced in a dextrose-glycine reaction mixture. In the absence of reducing sugars the reducing groups were not formed. The chemical nature of the reducing compounds indicates that they contain the enediol structure and that they may be classified as reductones (18). These results corroborate those of Hodge and Rist (18).

The concentration of fat antioxidants in the water extracts of the dextrose sugar cookies was not great, particularly when compared to the efficiency of the butylated hydroxyanisole to inhibit oxidative rancidity. This might be expected, since perhaps not all reducing compounds were extracted from the cookie and also because of the transitory nature of reductones in the browning reaction. Nevertheless, their concentration in the dextrose cookies appears to be sufficient to impart significant fat antioxidant effects, thus increasing cookie shelf life.

Acknowledgment

Acknowledgment is given to Corn Products Sales, Inc., for financial assistance in making this study possible.

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BROWNING REACTION PRODUCTS OF CAKE CRUMB[1]

PHILIP NORDIN AND JOHN A. JOHNSON[2]

ABSTRACT

The nature of the browning reaction products in cakes made with a high concentration of honey or reducing sugars has been investigated. Several of these browning-reaction products extracted from cake crumb have been separated by paper chromatography. One of these has an ultraviolet absorption maximum at 287–293 mμ. It rapidly reduces ammoniacal silver nitrate and 2,6-dichlorophenolindophenol in the cold and gives a deep blue color with aqueous ferric chloride. The ultraviolet spectrum and all other properties investigated show that the compound is not hydroxymethyl furfural or triose reductone. It is produced in the decomposition of 1-desoxy-1-piperidino-D-fructose and from the condensation products of glucose with phenylalanine and glycine. The compound browns rapidly with amino acids at a neutral pH and appears to be significant in the browning reaction.

Extensive studies on the browning or Maillard reaction (15) in recent years have dealt to a great extent with model systems of glucose and organic amines or amino acids. Browning with proteins is encountered in baking whenever reducing sugars, such as corn syrup, dextrose, invert, or honey, are used. Browning associated with these reducing sugars has been demonstrated to be of the Maillard type rather than caramelization. The phenomenon is accelerated in the presence of added amino acids (8).

From model systems, certain clear-cut reactions have been demonstrated. Crystalline N-glycosides of glucose with aromatic and aliphatic amines and their Amadori rearrangement products have been described. These reactions and their relationship to browning have been reviewed and restudied by Hodge (11, 12, 13). The Amadori rearrangement products upon heating undergo extensive degradation with the production of reductones, fluorescent compounds, and brown pigments (13). The amine base can be recovered in low yield, but glucose is not produced as it is from N-glycosides. It has been demonstrated by a large number of workers that glucose combines with amino acids. The noncrystalline products of Gottschalk (7) and Borsook (1) behave similarly to Amadori rearrangement products in that glucose cannot be recovered on hydrolysis. It is believed widely that browning with amino acids or proteins follows such an initial reaction. It has been

[1] Manuscript received August 27, 1956. Contribution No. 273, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. A report of work under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research Branch of the Agricultural Research Service. Presented at the 41st annual meeting, New York, May 1956.

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demonstrated that glucose combines with the amino groups of protein in the solid state (14, 10). Irreversible changes soon follow. The brown pigments that are formed cannot be dialyzed or readily removed from the protein (16). These facts are in agreement with the studies on model systems.

From knowledge of model systems, certain generalizations about the two initial steps of the browning reaction can be made. The first step is reversible and is favored by neutral or alkaline conditions. In bakery products such as cake with a neutral pH and high sugar content, conditions are favorable for the first step of the reaction. The Amadori rearrangement is, however, acid-catalyzed. As in the case of amino acids (1), the catalysis with protein could be provided by the carboxyl group.

In the decomposition of Amadori rearrangement products some fragmentation occurs with the liberation of nitrogen-free components. This report treats of the isolation of such sugar fragments from cake and the comparison of them with fragments obtained from the Amadori rearrangement products of the reaction of glucose with phenylalanine, glycine, and piperidine. Evidence is presented that the Amadori rearrangement may take place in the browning of glucose with protein during baking.

Materials and Methods

Cake Extract. A white pound-cake formula was used. Sucrose was replaced by glucose so as to cause browning, and the shortening was omitted to simplify the isolation procedure. A typical crumb pH was 6.8 to 7.3. The brown crust was cut away and only the center crumb, which had browned very little, was placed in a beaker and extracted several times with ethyl acetate. The ethyl acetate extract was concentrated in vacuum to a small volume. The concentrated extract was applied directly to filter paper and chromatographed in water-saturated n-butanol containing 1–2% acetic acid. Cakes prepared with sucrose were treated in like manner for comparison.

Browned Casein Extract. A mixture of 100 g. purified casein and 100 g. glucose was mixed with sufficient water to form a stiff dough. The dough ball was placed in an oven at 100°C. for 1½ hours. Browning throughout the dough ball was evident. The mixture was finely pulverized and extracted with ethyl acetate as described above for cake crumb.

Caramel Extract. An ethyl acetate extract was obtained from a glucose caramel prepared by heating a glucose syrup until a pronounced color was evident.

Amadori Rearrangement Products of Model Systems. 1-Desoxy-1-piperidino-D-fructose (DPF) was prepared according to the procedure of Hodge (13). Fructose-glycine was prepared according to the procedure of Borsook (1). Fructose-phenylalanine was prepared by the method of Gottschalk (7). This product was not completely homogeneous, as has also been reported by Gottschalk (7).

The degradation products of these preparations, on heating in aqueous solution, were examined by paper chromatography and compared with cake and protein extracts.

Hydroxymethyl Furfural. Hydroxymethyl furfural was prepared by the method of Haworth and Jones (9).

Triose Reductone. Triose reductone was prepared by the method of Castelfranchi (2), a modification of the procedure of Euler and Martius (5, 6).

Paper Chromatography. Whatman No. 1 filter paper was used. For examination of the decomposition products of the Amadori rearrangement products, a developing solvent of water-saturated n-butanol containing 1% acetic acid was used. For detection of amino acids, the chromatograms were sprayed with a 0.1% solution of ninhydrin in n-butanol. Reducing compounds were detected by spraying with ammoniacal silver nitrate and 2,6-dichlorophenolindophenol solution.

Results and Discussion

A 5% solution of DPF, adjusted to pH 6.8 with acetic acid, was heated at 90°C. and samples were withdrawn at various time intervals and chromatographed (Fig. 1). Cake crumb, caramel, and browned protein extracts are compared with a heated DPF solution in Fig. 2. The compound marked 3 was of interest because it appeared to be present in all the samples. Compound 3 possessed strong reducing properties, since it reduced ammoniacal silver nitrate in the cold, requiring from 1 to 5 minutes for the spot to develop. However, with the caramel extract the resolution was not good. Hence, the presence of compound 3 in the caramel extract could not be established. There were a number of fluorescent compounds in the protein extracts shown as open spots, not present in the heated DPF. It is difficult to evaluate the significance of these. However, the formation of compound 3 in fair yield from DPF and its apparent presence in browned protein extracts appeared to be significant. Accordingly, the fructose-phenylalanine and fructose-glycine preparations were heated in the same manner. In both cases, as well as with DPF, the formation of compound 3 occurred before visual browning was apparent. These compounds also yielded the elongated spot No. 4. This is apparently a mix-

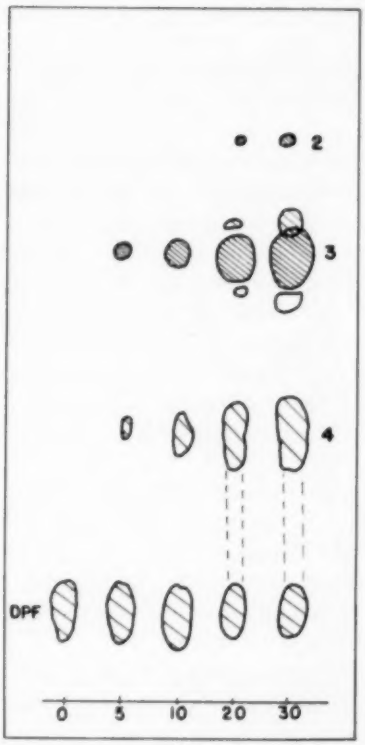

Fig. 1. The decomposition of 1-desoxy-1-piperidino-D-fructose (DPF). Abscissa, time of heating in minutes. 2 and 3 reacted rapidly with the silver nitrate spray. Dotted lines indicate a fluorescent streak.

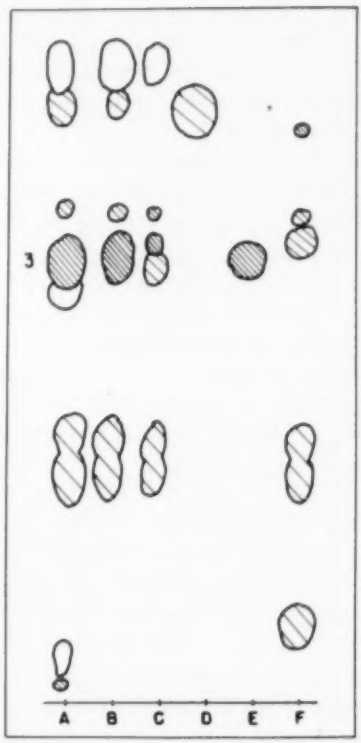

Fig. 2. Compound 3 from various sources. A, cake extract; B, glucose-casein extract; C, caramel extract; D, hydroxymethyl furfural standard; E, triose reductone standard; F, heated DPF solution.

ture of compounds that oxidize with difficulty. The presence of hydroxymethyl furfural (Fig. 2) in cake extracts was verified by spraying chromatograms with resorcinol in hydrochloric acid (7).

It also has been noticed that the pH of the solution is critical. If a solution of DPF is heated at pH 10, browning is extremely rapid. There is produced, in addition to compound 3, a true reductone, shown in Fig. 1 as spot No. 2. At pH 10, this compound was produced in higher yield than compound 3, whereas at pH 6.8 (Fig. 2) this reductone was present only in traces after 30 minutes of heating.

Compound 3 from the cake extract was obtained in homogeneous form by eluting the appropriate sections of several chromatograms with water and lyophilizing the extract. The compound was obtained as a dark oil (yield 40 mg. from 500 g. cake crumb). When rechromato-

graphed, traces of impurities were found. All attempts to crystallize this material failed.

Despite the low yield and noncrystalline character, certain definite information was obtained regarding the chemical properties of compound 3.

A water solution of the compound is weakly acidic. It could, however, be titrated to a definite phenolphthalein end point if the standard sodium hydroxide was added slowly with constant agitation over a period of 10 to 15 minutes. A definite stable end point was reached finally, corresponding to a neutralization equivalent of 190. Because of the syrupy nature of the product, this is undoubtedly high. The manner of the neutralization, however, indicates that if a carboxyl group is being titrated it must be bound, possibly as a lactone ring. However, it is more likely that enolic hydrogen is being titrated.

A nitrogen analysis was made by the micro diffusion method (3) and was negative.

The compound yields a deep-blue color with ferric chloride indicating enolic hydrogen. In this respect it is like triose reductone (4), produced by strong alkaline degradation of glucose. Note also the R_f of the two compounds are almost identical (Fig. 2). Compound 3 could be distinguished from triose reductone, however, by the fact that it gave no color reaction with aniline.

The compound is not so readily oxidized as triose reductone but it reduced 2,6-dichlorophenolindophenol. Acidic silver nitrate oxidized compound 3 very slowly. Fearon's *o*-dinitrobenzene test for enediols was negative.

The compound absorbs very strongly in the ultraviolet range of 287 to 293 $m\mu$ (Fig. 3). The spectrum is different from that of hydroxymethyl furfural, a compound frequently associated with browning. The R_f value in water-saturated n-butanol (Fig. 2) is also distinctly different from that of hydroxymethyl furfural.

The samples of compound 3 used to obtain these spectra (Fig. 3) were eluted from paper chromatograms and diluted with water so that all of them had approximately the same optical density at 290 $m\mu$. The positions of the maxima in all the samples are almost identical. They differ mainly in the height of the peak which occurs below 230 $m\mu$. The relative height of this peak, however, varies with the extent of browning and apparently is an impurity. This is illustrated by curves B and E (Fig. 3) obtained from two different samples of compound 3 from DPF. They differ only in that DPF had been heated for 30 minutes in the case of curve B and 10 minutes for curve E. The impurity has shifted the position of the maximum slightly.

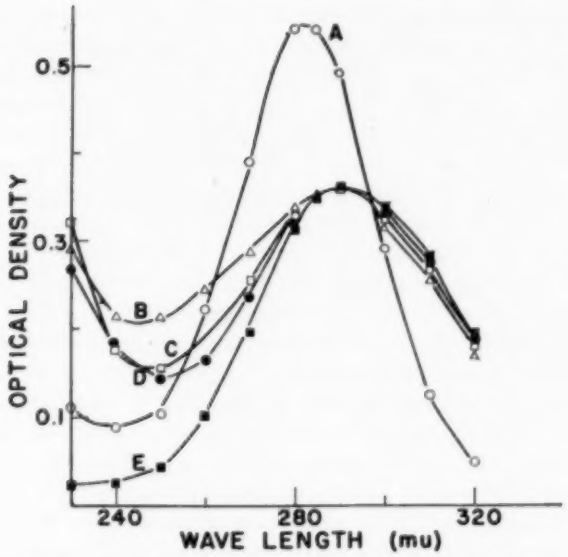

Fig. 3. Ultraviolet spectra of compound 3 as compared with hydroxymethyl furfural. A, hydroxymethyl furfural standard; B, compound 3 from DPF which was heated 30 minutes; C, compound 3 from glucose-casein extract; D, compound 3 from cake extract; E, compound 3 from DPF which was heated for 10 minutes.

The compound yielded a precipitate with 2,4-dinitrophenylhydrazine that could not be recrystallized to a constant melting point. Similarly, with benzoylchloride, a noncrystalline derivative was obtained. The latter was readily oxidized with aqueous potassium permanganate.

The compound is rapidly destroyed on heating at 100°C. in dilute alkali or with glycine. With amino acids extremely rapid browning took place at pH 7.0. Ten to 20 minutes of heating in the presence of glycine at pH 7.0 resulted in almost complete destruction of the compound. Paper chromatography revealed that a number of compounds were formed, some of which fluoresced.

Chichester, Stadtman, and Mackinney (3) in 1952 separated 24 components from a heated glucose-glycine mixture. Their chromatographic techniques were similar to those reported in this work. Compound 3 may be identical with compound 7 described by them.

Interest was attached to the compound referred to as compound 3 because it appeared to be produced through caramelization as well as through the Amadori rearrangement product. It was demonstrated that traces of amino acids catalyze its formation in glucose syrup with heating. Two tubes of 80% glucose syrup were heated at 100° C. To one tube was added a trace of glycine (molar ratio of glucose to glycine

100:1). One-milliliter samples were withdrawn at various time intervals and extracted with 10 ml. of ethyl acetate concentrated to dryness and dissolved in 0.5 ml. water. Examination by paper chromatography revealed that compound 3 was produced in the tube containing the amino acid after 15 minutes of heating, whereas in the glucose syrup it was not evident even after 2 hours of heating. From the work with synthetic Amadori rearrangement products, it appears that the catalysis is through this rearrangement and that the rearrangement takes place in glucose-protein systems such as occur in bakery products.

It was observed by Hodge (13) that DPF on heating yields free piperidine. It has been noted, chromatographically, that the formation of compound 3 occurs simultaneously with the formation of the free base. This is also true with the amino-acid condensation products. The free amino acid can be detected by spraying a chromatogram with ninhydrin. It was of interest to obtain quantitative data on the formation of compound 3. A 5-g. sample of DPF was dissolved in water and adjusted to pH 6.8 with acetic acid and made up to 50 ml. The solution was then heated at 85°C. Samples were withdrawn and spotted for chromatographic separation along with a standard containing compound 3. The remaining samples were diluted to 5 ml. with water and the optical densities measured at 500 mμ as a measure of color development. The chromatograms, after development, were sectioned and the portion containing the standard was sprayed with silver nitrate solution. Compound 3, the location thus revealed, was eluted with water and the optical density measured at 287 mμ. The data are presented in Table I.

TABLE I

MEASUREMENT OF THE DECOMPOSITION PRODUCTS OF DPF[a]

TIME	OPTICAL DENSITY OF ENTIRE SAMPLE (500 mμ)	OPTICAL DENSITY OF COMPOUND 3 OBTAINED FROM 6.7-μl. SAMPLE (287 mμ)
minutes		
0	0.0	0.0
3	0.001	0.0
6	.004	0.0
9	.003	0.01
12	.011	.035
15	.017	.055
18	.038	.087
21	.068	.115
24	.106	.155
27	.182	.167
30	.264	.190
33	.352	.230
36	.512	.270
39	0.650	0.275

[a] 1-Desoxy-1-piperidino-D-fructose.

It is evident that significant amounts of compound 3 are formed near the beginning of the heating and the amount increases gradually. Browning, however, is accelerated as heating is continued. This is shown graphically in Fig. 4.

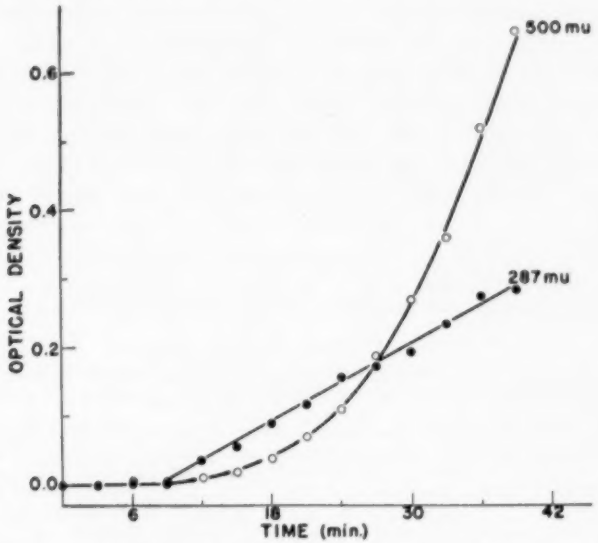

Fig. 4. Development of compound 3 during browning of DPF. Browning was measured at 500 mμ.

In another experiment various volumes of heated DPF were applied to paper, and after development the appropriate sections were eluted. Measurement at 287 mμ yielded the results reported in Table II.

When the results are plotted on a graph, an approximately linear relationship is found between optical density and volume of solution. The total errors of the method, largely pipetting, varied from 5 to 20%. The optical density readings at 287 mμ reported in Table I and

TABLE II

Optical Density of Compound 3 As a Function of Concentration

Volume of Heated DPF[a]	Optical Density of Eluted Compound 3 (287 mμ)
μl.	
0.0	0.0
6.7	0.101
13.4	.195
20.1	.350
26.8	.460
33.5	.600
40.2	0.730

[a] 1-Desoxy-1-piperidino-D-fructose.

Fig. 4 are thus proportional to the concentration of compound 3 in the sample.

Conclusions

The work reported here is evidence that, under bakery conditions, sugar amine condensation proceeds as postulated by many workers. An important stage after the Amadori rearrangement, however, is one where the nitrogen body is cleaved from the sugar fragment. Browning must result to a considerable extent when the highly reactive sugar fragment recombines in some way with the nitrogen-containing species. The composition of the sugar fragment referred to as compound 3 remains unknown. Characterization of this compound should prove interesting.

Acknowledgment

The authors are indebted to Dr. J. W. White, Jr., Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pa., for many helpful suggestions.

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EFFECT OF pH ON CAKE VOLUME AND CRUMB BROWNING[1]

DONALD MILLER, PHILIP NORDIN, AND JOHN A. JOHNSON[2]

ABSTRACT

Excessive browning of cake crumb may occur when high concentrations of honey or other sweetening agents containing reducing sugars are used. Excessive browning is accelerated by increase in pH, typical of the Maillard reactions. The undesirable effect can largely be eliminated by use of a leavening acid to maintain the pH of the crumb at approximately 6.3. The addition of a leavening acid, however, accelerated the loss of carbon dioxide during mixing of the cake batter. This resulted in partial loss of cake volume.

Loss of carbon dioxide during mixing was materially reduced and the pH of the cake crumb controlled by coating the leavening acid with hydrogenated vegetable oil. The acid released by heat during the latter stages of baking controlled the final pH and browning of the cake crumb.

Honey and dextrose are used widely in various types of baked goods. The amount and types of honey which can be used in bread, cake, sweet doughs, and cookies has been dealt with in previous publications from this laboratory (5, 6, 7, 8). Special problems arise, however, when honey is used in cake. First, it causes loss in volume which can be overcome by increasing the amount of soda leavener (6). However, increasing the amount of soda may cause excessive browning in the interior, especially in white cakes. This objection applies when any reducing sugars (honey, corn sugar, invert sugar) in moderate concentration are used. The role of the Maillard reaction in baked products has been discussed (2, 3, 4) and will not be dealt with here.

It is well known that the Maillard reaction is accelerated with an increase in pH. Thus in cake crumb, the amount of browning depends upon the final crumb pH. Most of the cake-crumb browning occurs near the end of the baking period, and considerable improvement in color results at the higher pH levels if the baking time is kept to a minimum. Cakes invariably brown more near the bottom of the pan and thus it is difficult to obtain a representative sample for reflectance studies, measurement of optical density, reducing value, or other changes generally associated with browning. In this study, the primary interest was in baking at a pH which was low enough to inhibit browning of the cake crumb in batters which contained high levels of reducing sugar. It has been observed repeatedly that with a crumb pH

[1] Manuscript received August 27, 1956. Contribution No. 254, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research Branch of the Agricultural Research Service. Presented at 40th annual meeting, St. Louis, Missouri, May 1955.

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of approximately 6.3 the visible effects of browning are insignificant. The volumes of the cakes, however, generally were lower with a drop in pH from neutral, so that baking in this pH region to inhibit browning was unsatisfactory. A study was therefore made to determine the cause of the poor volume and how it could be overcome while at the same time controlling the crumb color.

Materials and Methods

A white pound-cake formula was used in all experiments reported here. The composition was as follows:

	g.
Flour	200
Sucrose	100
Honey	125
Shortening	100
Egg white	120
Milk powder	20
Salt	6

All cakes were baked at 350°F. for 30 minutes. The amount and type of leavening agent were as described in the individual experiment.

In industry the activity of a baking powder is measured in a special apparatus (1). A standard batter is mixed in a closed container and the increase in volume of the batter and atmosphere is measured on a gasometer. In this work, it was desired to have a direct measure of the carbon dioxide that escapes from the batter. Also it was desired to have the same type of mixing as used in the baking laboratory and to be able to use the batter for subsequent baking. A rubber hood, there-

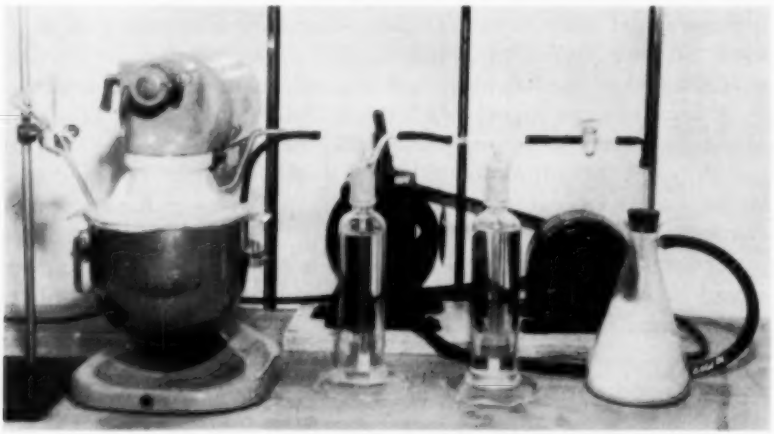

Fig. 1. Apparatus for measuring carbon dioxide escaping from the batter during mixing. The train includes two gas absorption bottles and a moisture trap containing calcium chloride.

fore, was fashioned for the Hobart mixer, Model N-50, so that the gases evolved during mixing could be collected and measured. Inlet and outlet connections were made on the rubber hood to permit air to be drawn over the batter as it was mixed. During operation, air was drawn continously and at a constant rate through the bowl. The outlet gases were passed through two gas-absorption bottles containing barium hydroxide solution (Fig. 1). The excess barium hydroxide was titrated with standard hydrochloric acid to a phenolphthalein end point as a measure of the carbon dioxide escaping from the batter.

A sequence of mixing was adopted as follows:

	Time involved (minutes)
1. Mix honey, sucrose, shortening, salt, milk powder	1
2. Add half the egg whites and continue mixing	5
3. Add flour (sifted with soda and leavening acid) and remainder of egg whites; attach hood, start mixer, and begin drawing air through mixing bowl	1
4. Add water slowly while mixing	2
5. Continue mixing	1
6. Stop mixer, but continue drawing air through bowl	11
7. Remove hood and weigh 6 oz. of batter into pans for baking	5

A coated potassium bitartrate was prepared by blending equal parts by weight of potassium bitartrate and hydrogenated vegetable oil (m.p. 150°F.). The material was heated until the fat melted and then was allowed to solidify while being stirred briskly. The solidified material was ground to particles fine enough to pass a 65-mesh screen. It was found that the addition of a small amount of n-butanol to the melted mixture produced a material that crumbled more readily after setting. The coating was more effective if the particles of leavener were coarse. The particle size reported above is an upper limit beyond which a mottled color effect was produced in the cake crumb, indicating local excesses of acid.

Results and Discussion

A series of batters was prepared with various levels of potassium bitartrate and each batter containing 2.5 g. of soda, incorporated by sifting with the flour. The carbon dioxide loss in mixing was determined for these batters by the procedure outlined. Six-ounce quantities of batter were subsequently weighed into pans and baked 30 minutes at 360°F. The volume and crumb pH[3] of these cakes were measured. These data are presented in Table I and Fig. 2.

Carbon dioxide losses were corrected for the volume of carbon dioxide in the air drawn over the batter, which was found to be 3.0 ml.

[3] Ten grams of cake crumb were dispersed in 50 ml. of water and the pH measured directly on the slurry.

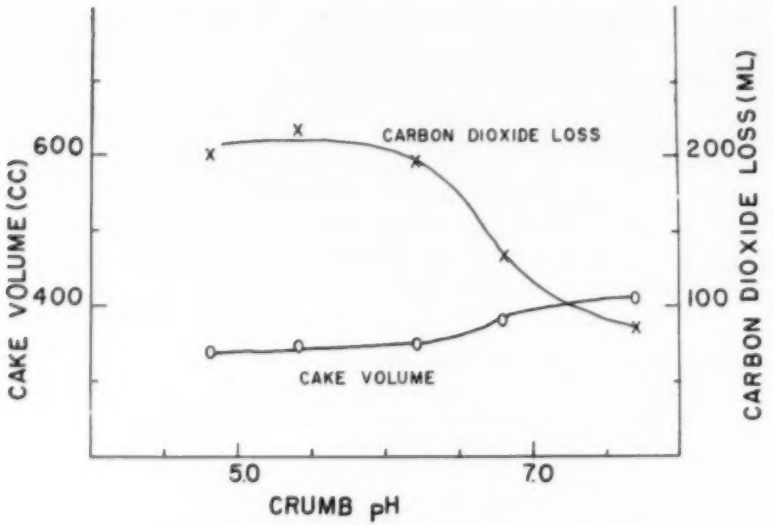

Fig. 2. The effect of crumb pH on carbon dioxide loss and cake volume.

TABLE I

EFFECT OF POTASSIUM BITARTRATE ON CAKE VOLUME, CARBON DIOXIDE LOSS, AND CRUMB pH

SAMPLE	POTASSIUM BITARTRATE	LOAF VOLUME	CARBON DIOXIDE LOSS	CRUMB pH
	g.	*ml.*	*ml.*[b]	
1 (Control)[a]	...	500	158	6.8
2	3	507	87	7.7
3	5	485	129	6.8
4	7	447	195	6.2
5	9	447	210	5.4
6	12	440	197	4.8

[a] Contained 10 g. of commercial baking powder with no additional soda.
[b] At 0°C. and 760 mm. pressure.

It can be seen that carbon dioxide loss increased in a pronounced manner with drop in crumb pH. The results reported above are, of course, highly empirical. It is necessary to standardize each step of the mixing process to obtain reproducible results. Although these data were determined for soda and potassium bitartrate, a similar but not so pronounced effect was observed when conventional baking powder was employed and the crumb pH was lowered with potassium bitartrate.

It has been pointed out that the crumb pH should be approximately 6.3 to effectively inhibit browning, but that this caused excessive premature loss of the carbon dioxide in the batter during mixing. Browning becomes marked, however, only in the latter stages of bak-

ing. An experiment was therefore devised in which regular baking powder was used in combination with coated potassium bitartrate. The coating was selected to melt only on heating and in time to liberate the acid to prevent browning. The most effective method devised to coat the acid was that already described. Its influence on volume was demonstrated in the following experiment:

White pound-cake batters were prepared containing the normal amount of baking powder plus an additional leavening acid. The formula used was that described under "Materials and Methods" except that the size of the batter was increased threefold. A batter without the honey and additional acid was used as control. The procedure in each case was to prepare the batter and then put 6-oz. samples into pans at various time intervals after mixing. The crumb pH was determined for the first and the last cake of each of the time series. These data are reported in Table II and Fig. 3.

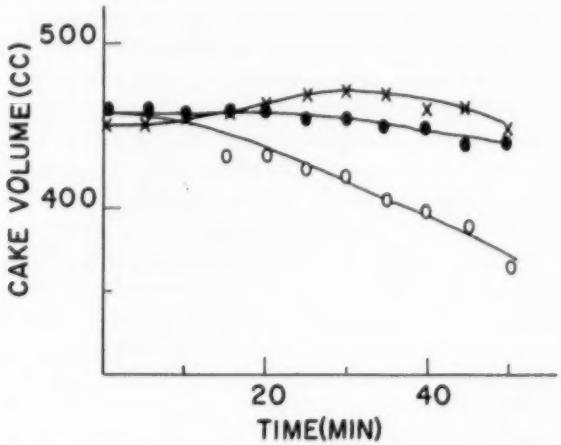

Fig. 3. Stability of cake batters. X, Sucrose standard with no honey; open circles, honey cake with coated potassium bitartrate; solid circles, honey cake with uncoated potassium bitartrate.

These data show that the sucrose batter at the pH level of 6.88 (Sample 1) did not deteriorate on standing, since the volumes of the cakes were essentially the same over a 50-minute resting period. Batter No. 2, pH 5.9, which contained the coated bitartrate, yielded similar results. In the cake batter that contained untreated bitartrate, the cake volumes became progressively lower with standing time (Sample 3). Undoubtedly, the size of the batter influences the rate of loss of carbon dioxide. The insignificant loss of volume at zero time shown by these data is believed to be due to the rather large batters employed.

TABLE II
STABILITY OF CAKE BATTERS

Time	Sample 1[a]		Sample 2[b]		Sample 3[c]	
	Cake Volume	Crumb pH	Cake Volume	Crumb pH	Cake Volume	Crumb pH
minutes	*ml.*		*ml.*		*ml.*	
0	450	6.88	460	5.90	460	5.85
5	450	...	460	...	460	...
10	455	...	455	...	460	...
15	455	...	460	...	430	...
20	465	...	460	...	430	...
25	470	...	455	...	430	...
30	470	...	455	...	420	...
35	470	...	450	...	405	...
40	460	...	450	...	400	...
50	450	6.88	445	6.00	365	...

[a] Standard containing sucrose and commercial baking powder.
[b] Honey cake with coated potassium bitartrate.
[c] Honey cake with uncoated potassium bitartrate.

Manufacturers of baking powder recognize that carbon dioxide loss during mixing must be minimized, even in neutral batters. This has been accomplished by the use of the moderately insoluble monocalcium phosphate, or a coated monocalcium phosphate (9). For cakes having an acid crumb, required to inhibit browning, the controlling of the loss of carbon dioxide is even more important. Although loss of carbon dioxide may be minimized in batters having an acid reaction by baking shortly after mixing, this is not always possible in commercial cake production. In cake production using honey, the control of the pH of crumb in the acid range is important, particularly in the later stages of baking when the temperature is high enough to accelerate the crumb browning. The use of the coated leavening acid provides the necessary acid, only late in the baking process, when it is required for the control of crumb browning.

Acknowledgment

The authors are indebted to Dr. J. W. White, Jr., Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania, and to Dr. B. S. Miller, Department of Flour and Feed Milling Industries, Kansas State College, Manhattan, Kansas, for their helpful suggestions.

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INFLUENCE OF VARIETY, FERTILIZER TREATMENT, AND SOIL ON THE PROTEIN CONTENT AND MINERAL COMPOSITION OF WHEAT, FLOUR, AND FLOUR FRACTIONS [1]

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ABSTRACT

The influence of variety, soil, and fertilizer treatment on wheat yield and on protein, total ash, and individual mineral elements in the ash of wheat and flour fractions was investigated in two plantings on Wooster silt loam soil. One planting was on low-fertility, acid soil; the other on well-limed fertile soil. Three varieties were grown, representing respectively the hard red winter (Pawnee), soft red winter (Seneca), and white (Cornell 595) wheat classes. Heavy applications of nitrogenous, phosphatic, and potassic fertilizers, singly and in all combinations, were made.

Milling behavior and flour yield were not noticeably affected by soil differences or fertilizer treatments. Applications of nitrogen in some cases increased the percent protein in wheat and flour. Total wheat ash was different among the varieties, and was affected by fertilizer treatments.

Potassium made up a higher percentage of all ashes than any other element. It was highest in the water-solubles fraction of the flour, and lowest in the gluten. Magnesium had much the same pattern of distribution, but differences were much smaller. Manganese was highest in the ash from water-solubles, and lowest in the starch.

Gluten ash showed relatively high percentages of iron, zinc, aluminum, calcium, copper, and tin. Lead and molybdenum were detected only in gluten and starch ashes. Silicon, boron, and silver were fairly uniformly distributed throughout the kernel.

The percentage of specific elements in the ashes of the wheat and the flour fractions varied greatly between varieties for some elements. Between varieties there was no consistent relationship among elements compared as absolute amounts or as percentages. This probably indicates a complex hereditary background influencing the composition of the ash.

The mineral elements play an important role in plant growth and development, both directly as essential nutrients and indirectly

[1] Manuscript received May 24, 1956. Contribution from the Department of Agricultural Biochemistry, Ohio State University, Columbus, and the Department of Agronomy, Ohio Agricultural Experiment Station, Wooster. Paper No. 36-56 of Journal Series, the Ohio Agricultural Experiment Station. Part of a dissertation submitted to the Graduate School of The Ohio State University by M. M. El Gindy in partial fulfillment of the requirements for the degree, Doctor of Philosophy, May, 1954.
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through their effect on enzyme systems. The behavior of doughs prepared from wheat flour is probably influenced by the cations present, especially those in the protein molecules. It was suspected that the amount and proportion of cations present in the wheat grain might vary with variety, soil, soil treatment, and season. The present study was set up to determine whether such differences occur.

There is an extensive literature on the chemical composition of wheat, flour, and mill feeds. In many cases the analyses were made on samples from desultory and in some cases unknown sources. Data on soil, yield level, climatic factors, and variety are frequently lacking. Results indicate that protein, total ash, and mineral elements of the ash may vary greatly.

Early work at the Ohio Station by Ames and co-workers (2, 3, 4) indicated that added fertilizers did influence mineral composition of wheat ash. Gericke (7, 8, 9) showed that the amount of available nitrogen in the soil influenced the protein content of wheat. He also demonstrated with wheat grown in tank culture that the chemical composition of the grain varied depending on the stage of growth at which mineral nutrients were available to the plants. He also found variety differences. Bailey (6) gives an excellent review of the literature up to 1944. Schrenk (11) reports three- and in some cases four-year analyses on three varieties of wheat grown at 14 places in Kansas. Variety differences in composition of grain ash are not discussed, and examination of the tables of analytical data does not indicate that significant differences existed. Single samples were analyzed in all cases. Schrenk cites the more important literature up to 1955.

Materials and Methods

The Pawnee (hard red winter), Seneca (soft red winter), and Cornell 595 (white) varieties were grown at two nitrogen, two phosphorus, and two potassium levels, in all combinations, for two seasons. The nitrogen application was 42 lb. per acre, 12 lb. in the fall and 30 lb. in the spring; phosphorus, 96 lb. phosphorus pentoxide per acre in the fall; and potassium, 96 lb. potassium oxide per acre in the fall. Including the unfertilized treatment there were eight combinations. The soil was Wooster silt loam both seasons, but in 1951 the plots were located on an unlimed area of low fertility, and in 1952 on a productive field that had been limed, well fertilized, and cropped in a good rotation for many years. The two seasons were much alike, and the prime difference between the two crops was due to soil conditions. For this reason the factor will be referred to as "soil" rather than "season."

The field experiment was set up in duplicate. Each sample analyzed was a composite from two plots. For determinations on whole grain, the wheat was ground in a small Wiley mill, using a 40-mesh screen. Larger samples were milled on an Allis-Chalmers experimental mill, and a straight flour prepared. The flours were fractionated into gluten, starch, and water-solubles by the method developed by Yamazaki (12). The separation was not complete. Most of the gluten was concentrated in the sample so designated, but undoubtedly a small amount of the starch was not removed, and some water-solubles may have been included also. The starch likewise contained fragments of gluten and small amounts of solubles not washed out. The water-solubles were decanted off after centrifugation and they too were likely contaminated. Nevertheless, separations were reasonably complete and marked differences in composition of ash were certainly associated with real differences between fractions.

Moisture determinations on the ground grain were made by the A.O.A.C. official method (5). Moisture in flour, gluten, and starch was determined by the standard aluminum plate method outlined in *Cereal Laboratory Methods* (1), modified by using a 30-minute period for drying. In the lyophilized water-solubles fraction, moisture was assumed to be 4% in all samples. This figure was determined by Yamazaki (12), and was used because water-solubles fractions apparently undergo chemical changes in oven drying.

Ash was determined without the use of any ashing aids. Samples were ashed in nickel crucibles at 525°C. Higher temperatures resulted in the loss of some of the phosphorus and potassium.

Total nitrogen was determined by the Kjeldahl method, A.O.A.C. (5), modified by distilling the ammonia into saturated boric acid solution. Protein was estimated as $N \times 5.7$.

Mineral analyses were made on whole wheat and the flour fractions by spectrographic means. The method used was developed by Sayre (10). The spectrograph was a Bausch & Lomb medium quartz instrument equipped with a 3-mm. two-step sector with a 5 to 1 ratio. Four-by-ten-inch SA No. 2 spectrographic plates were used. The samples were arced dry between carbon electrodes with approximately a 0.9-mm. gap. Excitation was by a 2200-volt A.C. arc at about 2.3 amperes. Line density determinations were made with a Jarrell-Ash recording microphotometer.

Lithium fluoride was used for the internal standard. Enough of the sample for analysis was weighed out to provide 10 mg. ash. To this were added 90 mg. lithium fluoride. The materials were mixed

and ashed. The resulting 100 mg. of ash plus buffer were subjected to spectrographic analysis. The ratio of standard to ash was 9:1.

An attempt was made to get a quantitative estimate of the amounts of 15 elements: phosphorus, potassium, magnesium, silicon, manganese, iron, zinc, aluminum, calcium, copper, tin, boron, silver, lead, and molybdenum. A synthetic ash was prepared and working curves constructed to convert the relative intensities into absolute concentrations. The method worked satisfactorily except for phosphorus. The density of the phosphorus lines of the samples was obviously much higher than that obtained from the synthetic ash, and the density varied little with the amount present. Unfortunately there were no residues from many of the samples, and it was not possible to salvage information. Phosphorus was therefore dropped from consideration.

Results

Wheat Yield and Protein. Wheat yield and protein content are presented in Table I. Yields in 1951 were low, averaging about 20 bu. per acre without fertilizer. Response to fertilizer was good. Phosphorus appeared to be the primary limiting element. When phosphorus had been supplied, then there was response to nitrogen. There

TABLE I

EFFECT OF VARIETY, FERTILIZER TREATMENT, AND SOIL ON WHEAT YIELD AND PROTEIN CONTENT

Fertilizer Used	Pawnee		Seneca		Cornell 595		Average Three Varieties	
	1951	1952	1951	1952	1951	1952	1951	1952
	Yield — bushels per acre							
None	19.4	32.2	20.5	43.5	20.2	45.0	20.0	40.2
Nitrogen (N)	18.1	49.8	25.1	53.0	22.9	47.6	22.0	50.1
Phosphorus (P)	25.3	33.4	26.5	50.1	27.9	41.2	26.6	41.6
Potassium (K)	22.2	36.6	26.8	49.8	21.9	45.8	23.6	44.1
N and P	29.1	43.9	29.4	56.0	36.1	55.3	31.5	51.7
N and K	24.8	51.0	28.3	54.7	30.1	57.0	27.7	54.2
P and K	20.1	35.3	24.6	44.0	30.9	51.3	25.2	43.5
N, P, and K	28.9	48.6	36.3	54.1	34.9	48.5	33.4	50.4
Av.	23.5	41.4	27.2	50.7	28.1	49.0	26.3	47.0
	Percent protein in wheat							
None	11.4	10.9	9.8	11.3	9.7	9.6	10.3	10.6
N	11.3	12.8	9.6	11.1	9.5	10.0	10.1	11.3
P	11.0	11.6	9.8	11.4	9.6	9.8	10.1	10.9
K	11.2	11.3	9.5	10.6	9.1	9.6	9.9	10.5
N and P	11.5	12.4	10.1	12.0	10.0	11.0	10.5	11.8
N and K	11.3	12.4	10.2	11.6	10.0	10.2	10.5	11.4
P and K	11.7	11.0	10.5	11.0	9.3	9.1	10.5	10.4
N, P, and K	11.3	12.3	10.0	10.9	9.1	10.2	10.1	11.1
Av.	11.3	11.8	9.9	11.2	9.5	9.9	10.2	11.0

was little response to potassium in any combination. Effect of fertilizers on percentage protein in the grain was small, even for nitrogen. In fact there was some tendency to lower protein percentage with higher yield, indicating insufficient nitrogen for the larger crop.

Wheat yields in general were perhaps 10% higher at Wooster in 1952 than in 1951. On the more fertile soil used for the experiment in 1952, yields were double those obtained from the poorer soil in 1951. Responses to nitrogen were evident in both yield and percentage protein in the grain. Because of adequate residual supplies in the soil

TABLE II

RECOVERY OF GLUTEN, STARCH, AND WATER-SOLUBLES (DRY WEIGHT) FROM FRACTIONATING 100 G. FLOUR (14% MOISTURE)

FERTILIZER USED	PAWNEE		SENECA		CORNELL 595	
	1951	1952	1951	1952	1951	1952
	Grams dry gluten from 100 g. flour (14%)					
None	11.4	12.7	11.7	13.9	10.0	10.2
Nitrogen (N)	13.8	17.5	11.9	14.8	10.2	10.4
Phosphorus (P)	11.6	14.5	9.4	14.2	8.6	8.9
Potassium (K)	11.9	12.4	10.3	14.1	9.3	8.6
N and P	13.6	16.8	9.9	15.7	10.3	12.0
N and K	12.6	17.4	9.9	15.7	9.9	10.2
P and K	12.1	12.6	7.2	13.0	7.8	9.3
N, P, and K	12.3	14.5	7.4	13.1	9.2	10.9
Av.	12.4	14.8	9.7	14.3	9.4	10.1
Av. 2 years	13.6		12.0		9.8	
	Grams dry starch from 100 g. flour (14%)					
None	65.2	62.2	64.9	60.6	65.8	65.0
N	66.7	59.3	62.9	62.9	66.8	65.5
P	66.9	61.8	67.4	61.9	67.4	66.0
K	67.4	61.7	67.7	64.3	67.4	64.3
N and P	66.8	60.0	65.3	61.8	66.7	64.4
N and K	66.4	58.1	64.6	62.2	67.2	66.9
P and K	66.1	63.0	67.0	64.1	66.7	67.3
N, P, and K	67.4	61.1	66.6	64.2	68.7	66.2
Av.	66.6	60.9	65.8	62.8	67.1	65.7
Av. 2 years	63.8		64.3		66.4	
	Grams dry water-solubles from 100 g. flour (14%)					
None	4.4	4.9	3.7	3.4	4.0	4.7
N	4.3	3.8	3.4	3.4	4.5	4.7
P	4.3	3.4	4.0	3.7	4.5	4.7
K	4.3	5.4	3.9	3.3	4.3	3.2
N and P	4.3	4.2	4.0	3.6	4.8	4.2
N and K	4.5	4.8	4.0	3.2	4.5	4.1
P and K	4.0	4.5	4.7	3.6	4.3	4.0
N, P, and K	4.0	3.8	4.7	3.4	4.4	4.0
Av.	4.3	4.4	4.1	3.5	4.4	4.2
Av. 2 years	4.4		3.8		4.3	

there was little direct response to phosphorus. Potassium increased yields moderately.

On these two soils the crop reacted differently to fertilizer treatments both in yield and protein in the grain. In some cases lack of response was apparently due to an adequate supply already in the soil, and in other cases to some nutrient being limiting. The fertility level and lime level were very different in the two years. If the amount or composition of the ash is affected by these factors, the range provided should have been enough to indicate something of the magnitude and character of the influence.

Fractionation of Flours. Recovery of gluten, starch, and water-solubles is given in Table II. The amount of gluten recovered from a sample was largely a function of the amount of protein present. As expected, the hard red winter wheat Pawnee was highest in protein and in gluten, and the white wheat Cornell 595 lowest.

Starch varied inversely with the gluten, since these two fractions together made up all but 3 or 4% of the flour. The proportion of water-solubles was fairly constant, although Seneca appeared to be somewhat lower than the other varieties. There were no obvious differences associated with fertility or treatment.

The weight of recovered dry matter in the fractions was about 7% less than that of the flour. This was a large loss, but unavoidable with the small flour samples. The most important sources of loss were: gluten sticking to the screens used during the washing process and to the hands of the operator, and adherence of material to the cups used in

TABLE III

WHEAT REQUIRED TO OBTAIN 100 G. STRAIGHT FLOUR; AMOUNT OF GLUTEN, STARCH, AND WATER-SOLUBLES OBTAINED; TOTAL ASH AND TOTAL PROTEIN IN WHEAT AND FLOUR FRACTIONS[a]

	Variety		
	Pawnee	Seneca	Cornell 595
	g.	g.	g.
Wheat required to produce 100 g. straight flour (both 14% moisture)	136.0	141.0	137.0
Dry gluten recovered	13.6	12.0	9.8
Dry starch recovered	63.8	64.3	66.4
Dry water-solubles recovered	4.4	3.8	4.3
Total ash in wheat	2.21	2.37	2.26
Total ash in gluten	0.07	0.05	0.04
Total ash in starch	0.18	0.17	0.18
Total ash in water-solubles	0.20	0.20	0.23
Protein in wheat	15.7	14.9	13.3
Protein in gluten	7.2	5.7	5.0
Protein in starch	0.6	0.7	0.9
Protein in water-solubles	0.8	0.9	1.0

[a] Flours as milled averaged about 12.7% moisture. The flour yield, corrected to 14% moisture, averaged 72.5%, 70.0%, and 72.0% for Pawnee, Seneca, and Cornell 595, respectively.

centrifuging the starch. Losses did not affect the composition of the samples, and therefore had no serious effect on the analyses of the ashes. It is possible that the amount of gluten recovered from the hard wheat was a little higher, because it was easier to form a dough from this flour and the gluten cohered better while washing out the starch.

Protein and Ash Content of Wheat and Flour Fractions. Table III

TABLE IV

AMOUNT OF ELEMENTS IN THE ASH FROM WHEAT REQUIRED TO PRODUCE 100 G. STRAIGHT FLOUR, AND IN THE FRACTIONS OF THE FLOUR OBTAINED; ALSO COMPOSITION OF EACH ASH STUDIED

Elements in Ash	Distribution of Ash Elements				Composition of Ash			
	Whole Wheat	Gluten	Starch	Water-Solubles	Whole Wheat	Gluten	Starch	Water-Solubles
	mg.	*mg.*	*mg.*	*mg.*	%	%	%	%
	Pawnee (136 g. wheat)							
Potassium	462.00	3.82	22.18	73.54	20.9	5.8	12.2	36.6
Magnesium	177.00	3.64	13.14	19.26	8.0	5.5	7.2	9.6
Silicon	33.97	1.21	1.77	2.22	1.5	1.8	1.0	1.1
Manganese	16.20	0.16	0.10	0.98	0.73	0.24	0.05	0.49
Iron	5.73	1.16	0.24	0.20	0.26	1.76	0.13	0.10
Zinc	4.58	0.38	0.17	0.16	0.21	0.58	0.09	0.08
Aluminum	2.84	0.26	0.55	0.25	0.13	0.39	0.30	0.12
Calcium	2.88	0.43	0.40	0.39	0.13	0.65	0.22	0.19
Copper	1.12	0.10	0.02	0.07	0.05	0.15	0.01	0.04
Tin	1.13	0.11	0.17	0.11	0.05	0.17	0.09	0.06
Boron	0.58	0.01	0.04	0.03	0.03	0.02	0.02	0.02
Silver	0.51	0.04	0.05	0.05	0.02	0.03	0.03	0.03
	Seneca (141 g. wheat)							
Potassium	556.00	2.77	23.16	67.13	23.5	5.4	13.6	34.2
Magnesium	228.00	2.23	8.69	11.60	9.6	4.4	5.1	5.9
Silicon	50.70	0.83	1.75	1.25	2.1	1.6	1.0	0.7
Manganese	33.56	0.22	0.31	1.94	1.42	0.43	0.18	0.99
Iron	8.04	1.01	0.36	0.24	0.34	1.98	0.21	0.12
Zinc	3.49	0.31	0.18	0.14	0.15	0.61	0.11	0.07
Aluminum	3.37	0.23	0.38	0.21	0.14	0.45	0.22	0.11
Calcium	2.64	0.42	0.36	0.33	0.11	0.82	0.21	0.17
Copper	1.33	0.07	0.03	0.11	0.06	0.14	0.02	0.06
Tin	1.08	0.10	0.15	0.09	0.05	0.20	0.09	0.05
Boron	0.61	0.01	0.05	0.04	0.03	0.03	0.03	0.02
Silver	0.51	0.01	0.04	0.05	0.02	0.02	0.02	0.03
	Cornell 595 (137 g. wheat)							
Potassium	688.00	2.38	26.70	77.91	30.4	5.4	14.8	34.2
Magnesium	192.00	1.98	8.47	13.40	8.5	4.5	4.7	5.9
Silicon	40.57	0.61	2.09	1.75	1.8	1.4	1.2	0.8
Manganese	21.71	0.24	0.30	2.83	0.96	0.55	0.17	1.24
Iron	3.49	0.86	0.35	0.28	0.15	1.95	0.19	0.12
Zinc	3.12	0.31	0.19	0.15	0.14	0.71	0.11	0.07
Aluminum	2.94	0.18	0.48	0.33	0.13	0.41	0.27	0.14
Calcium	2.67	0.51	0.37	0.31	0.12	1.16	0.21	0.14
Copper	1.44	0.07	0.04	0.25	0.06	0.16	0.02	0.11
Tin	1.05	0.12	0.17	0.11	0.05	0.27	0.09	0.05
Boron	0.58	0.01	0.08	0.06	0.03	0.03	0.04	0.02
Silver	0.49	0.02	0.05	0.06	0.02	0.05	0.03	0.03

gives the average amount of wheat required to produce 100 g. straight flour for each of the varieties; the average amount of gluten, starch, and water-solubles recovered on fractionation; and the average ash and protein content of the wheat and flour fractions. Each figure is the average of 16 independent determinations; only variety differences are shown.

Of the nitrogen in the wheat, approximately half was recovered in the gluten; most of the remainder went to the mill feeds; only minor amounts were found in the starch and water-solubles. In the starch, the nitrogen probably occurred in the tailings rather than in the prime starch, and also possibly in some gluten not removed.

Table IV gives mg. of each element in the ash from the whole wheat and from each flour fraction, based on the amount of wheat required to yield 100 g. straight flour at 14% moisture. It also gives the percent composition of each ash. Elements are arranged in the order of their amounts in the whole wheat. Each figure in the table is the average of 16 independent determinations.

Analysis of variance was made for protein and total ash and for each of the 14 elements studied. Each analysis included 48 independent determinations – three varieties for two "soils" with eight fertilizer treatments. The second-order interaction was used as the error term. Selected F values are given in Table V. These F values are proportional to the variances and therefore indicate roughly the relative influence of the factors.

Protein in the wheat was influenced by variety, soil, and treatment. Each of these and the variety × season interaction were statistically highly significant, but variety had much the largest variance, and appeared to be the dominant factor. Variety differences were to be expected because of the choice. Soil × treatment interaction was significant at the 5% level.

Protein in the gluten varied with variety and soil. The variety × soil interaction was also significant, but not very large. The fact that one soil was acid, the other well limed may partially explain why soil had a significant effect, but fertilizer treatment did not.

Protein in both starch and water-solubles was low. Nevertheless soil apparently influenced protein in starch, and variety influenced protein (or nitrogen) in the water-solubles. These results may have been partially due to better coherence of the higher protein glutens in the fractionation in 1952. Variety had a minor influence on protein in starch, but the variety × soil interaction was relatively large.

The amount of ash in the whole wheat was influenced by variety more than anything else. Treatment effect was considerably less but

still highly significant. Ash in the gluten varied between varieties, but was most strongly affected by soil. Soil also exerted the major apparent influence on total ash of both starch and water-solubles. The total ash of all flour fractions depended in part on the milling, and soil effects may have had little real significance. Total ash in all flour fractions showed little difference between varieties.

TABLE V

SELECTED F VALUES FROM ANALYSES OF VARIANCE FOR PROTEIN, TOTAL ASH, AND FOURTEEN ELEMENTS IN WHOLE GRAIN AND GLUTEN, STARCH, AND WATER-SOLUBLES FRACTIONS

	Analysis for							
	Variety	Soil	Treatment	Variety × Soil	Variety	Soil	Treatment	Variety × Soil
5% F	3.74	4.60	2.77	3.74	3.74	4.60	2.77	3.74
1% F	6.51	8.86	4.30	6.51	6.51	8.86	4.30	6.51
	F	*F*	*F*	*F*	*F*	*F*	*F*	*F*
	Whole wheat				Starch			
Protein	150.07**	73.88**	6.38**	10.04**	4.14*	18.63**	1.05	10.25**
Total ash	12.37**	0.19	5.50**	3.00	6.00*	16.00**	1.00	4.00*
Potassium	41.21**	5.14*	0.39	14.37**	1.41	3.87	0.70	0.19
Magnesium	2.40	7.44*	0.32	3.24	42.65**	0.37	1.73	8.32**
Silicon	5.08*	4.28	1.04	10.60**	1.33	0.00	0.69	0.13
Manganese	7.51**	74.74**	1.08	0.02	68.03**	8.04*	3.08*	3.32
Iron	0.38	48.51**	7.50**	52.32**	3.75*	1.45	0.89	2.23
Zinc	49.58**	2.12	1.65	5.86*	1.37	2.30	0.65	3.53
Aluminum	0.53	0.01	1.46	1.94	1.05	1.89	0.62	1.08
Calcium	2.00	14.00**	1.27	2.91	2.86	10.28**	8.14**	1.14
Copper	5.63*	16.85**	1.43	9.54**	37.80**	53.20**	1.40	13.80**
Tin	38.42**	16.33**	1.08	6.83**	0.24	0.05	0.84	0.09
Boron	0.39	22.62**	1.23	1.02	4.98*	0.34	1.08	1.46
Silver	6.23*	0.98	1.37	0.49	1.02	34.57**	1.74	2.07
Lead	..	..	..	..	0.21	115.60**	1.83	0.74
Molybdenum					0.37	3.59	1.09	1.79
	Gluten				Water-solubles			
Protein	37.39**	61.87**	1.83	4.92*	45.22**	2.92	2.46	0.27
Total ash	15.00**	20.00**	2.64	5.36*	4.92*	11.72**	0.39	0.56
Potassium	0.11	1.55	1.22	0.55	0.54	0.15	2.58	0.68
Magnesium	8.20**	21.24**	0.89	1.12	28.60**	13.95**	1.02	0.21
Silicon	0.40	18.84**	2.69	2.52	2.33	5.78*	1.20	1.76
Manganese	11.50**	0.57	0.74	1.14	29.16**	11.61**	1.56	1.86
Iron	0.18	6.79*	1.62	2.75	0.37	0.69	1.21	1.98
Zinc	3.12	11.27**	1.17	7.71**	2.28	9.12**	2.82*	7.10**
Aluminum	1.96	2.89	0.57	3.57	0.90	1.72	0.83	0.47
Calcium	17.90**	25.11**	1.41	23.94**	4.50*	1.10	0.47	0.73
Copper	1.81	8.07*	1.13	1.37	27.49**	59.36**	1.22	15.64**
Tin	16.66**	2.70	1.26	7.33**	2.61	0.29	0.63	0.15
Boron	2.58	0.04	0.74	0.62	15.54**	12.18**	5.10**	0.74
Silver	7.35**	0.90	2.01	4.33*	2.54	0.05	1.85	1.83
Lead	20.67**	10.62**	0.72	13.79**	0.92	1.36	0.80	0.50
Molybdenum	0.65	0.21	0.79	0.07	..	..	..	..

Individual Elements in the Ashes. A study of Table V will give a general idea of the importance of variety, soil, and fertilizer treatment in influencing the amount of the various elements in the ash of the whole wheat, and that of the flour fractions. Obviously environment has an important effect, but inheritance also plays a role.

In whole-wheat ash, potassium, silicon, manganese, zinc, copper, tin, and silver differed significantly between varieties. In gluten ash, variety differences in magnesium, manganese, calcium, tin, silver, and lead reached the level of significance. In starch ash, magnesium, manganese, iron, copper, and boron; and in the water-solubles ash, magnesium, manganese, calcium, copper, and boron were significantly different among varieties.

Percentagewise silicon, iron, zinc, aluminum, calcium, copper, tin, and lead were higher in gluten ash than in other flour fractions, or in the whole grain. Potassium, magnesium, and manganese were highest in the water-solubles; manganese, however, was higher in the whole wheat than in any flour fraction. The heavy elements were relatively high in the gluten ash.

Potassium was very high in the water-solubles ash, being roughly three times as high as in the starch ash, and six times the gluten ash level. Magnesium followed the same pattern, but differences between fractions were much less. Iron, zinc, calcium, and copper were three to 15 times higher in gluten ash than in that from other flour fractions. In spite of the relatively small amount of gluten ash, there was more actual iron, zinc, and calcium in the gluten than in either other flour fraction—and for iron, more in the gluten than in all the rest of the flour (see Table IV).

Variety was not the only factor influencing ash composition. Soil exerted a definite effect. For whole-wheat ash, potassium, magnesium, manganese, iron, calcium, copper, tin, and boron all differed significantly between the two crops. In the gluten ash, significant differences in magnesium, silicon, iron, zinc, calcium, copper, and lead were noted. In starch ash, manganese, calcium, copper, silver, and lead; and in the water-solubles ash, magnesium, silicon, manganese, zinc, copper, and boron, were significantly different for the two plantings.

Fertilizer treatments had definitely less effect on ash composition than variety or soil. Differences reached the level of significance for iron in the whole-wheat ash, for manganese and calcium in the starch ash, and for zinc and boron in the water-solubles ash. The F values were generally low, and in only three cases reached the 1% level of significance. Apparently the soil differences, including marked difference in pH level, had a greater effect on uptake and distribution of

mineral nutrients than did heavy applications of nitrogen, phosphorus, and potassium.

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EFFECT OF BAKING ON THE NUTRITIVE VALUE OF PROTEINS IN RYE BREAD WITH AND WITHOUT SUPPLEMENTS OF NONFAT DRY MILK AND OF LYSINE[1]

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ABSTRACT

The protein quality of rye bread and of the unbaked ingredients, containing 0 and 6% nonfat dry milk and 0.166% l-lysine, was determined by rat-growth studies. Supplements of milk solids increased the protein efficiency ratio 9% for the bread and 11% for the unbaked ingredients. Lysine increased the protein efficiency 21% both for the bread and the unbaked ingredients. The protein quality of the breads was lower than that of the unbaked ingredients.

Although rye bread is a familiar food in the United States, and rye is a very important bread flour in north-European countries, little study has been made of the protein quality of rye as compared with other cereal grains. French and Mattill, in 1935 (2), found the biological values of rye and wheat bread to be the same—about 74, as compared with 100 for milk. Sure, in 1954 (7), studied the relative nutritive values of the proteins in whole wheat and whole rye and the effect of amino acid supplements. He found the protein efficiency ratio of whole-rye flour to be 1.40 as compared with 0.92 for whole-wheat flour when fed at a protein level of 8%.

Since the addition of nonfat dry milk to wheat flour and bread improves the protein quality of the wheat, it seemed of interest to compare the protein quality of rye bread with and without supplements of milk solids. In the work reported here, the protein quality of rye bread ingredients and of rye bread containing 0 and 6% nonfat dry milk and 0.166% l-lysine was determined by rat-growth studies.

Materials and Methods

The protein quality of the rye breads was determined by the rat-growth method of Osborne, Mendel, and Ferry (5).

Diets. Three rye breads were made from the same lot of Klamath rye grown in Oregon and containing 10.2% protein on a moisture-free basis. The bread formula was: finely ground rye meal, 100%; salt, 2%; sugar, 2%; hydrogenated shortening, 2%; yeast, 2%; nonfat dry milk, 0 or 6%, or 0.166% l-lysine added as the monochloride of l (+) lysine.

[1] Manuscript received July 23, 1956. Department of Home Economics, University of California, Berkeley, California. The data in this paper are taken from a thesis presented to the graduate school of the University of California, Berkeley, by Anne-Sofie Strømnaes in partial fulfillment of the requirements for the degree of Master of Science, June 1955.

The sample of heat-treated, spray-dried, nonfat dry milk analyzed 2.77% lysine, by microbiological assay, from the acid hydrolysate of the milk. The assay was effected by the use of *Leuconostoc mesenteroides* P-60 with an incubation period of 72 hours at 37°C. A complete basal medium was used.[2] The lysine and nonfat dry milk were incorporated with the flour. The doughs made from 200 g. of flour were fermented for 2 hours and baked for 35 minutes at 430°F. The bread was cut into small pieces, dried in a dehydrator at a temperature not over 122°F., and ground very fine.

Two per cent fat and 2.5% Hubbel salts were included in the final diets. The nitrogen content of the diets was equalized by diluting them with corn starch to a nitrogen content of 1.42±0.04% determined by the basic rye bread diet.

In the unbaked diets, the dry ingredients were thoroughly blended and ground very fine. The shortening was added last. An equivalent amount of active-dry yeast which had been heated at 110°C. for 15 minutes was used in place of the compressed yeast.

Feeding and Experimental Procedure. Details of the selection and care of the rats were essentially the same as described in a previous paper (6). For the 2-day adjustment period, a basal diet containing 6% casein was given. All experimental diets were fed *ad libitum* for 5 weeks. Vitamin supplements (as in experiments 3, 4, 5, and 6 of the previous work) were given three times a week.

Results and Discussion

Comparison of Protein Quality of Rye and Whole Wheat. The protein efficiencies for rye bread and for the unbaked ingredients, 1.97 and 2.17, respectively (Table I), were much higher than those for whole-wheat water bread and for the unbaked ingredients, 1.17 and 1.49, respectively (6). Although the percentage of protein differed in the diets—8% for the rye diets and 11% for the whole wheat, the data of Sure (8) showed that the percentage of protein in whole-wheat diets did not greatly alter the protein efficiency ratio, 0.90 with 5% protein as compared with 1.05 for 9% protein. This higher protein efficiency for rye as compared with wheat was reported by Sure (7) in his studies on whole-wheat and whole-rye flour, and by Kon and Markuze for wheat and rye breads (4). The latter workers found protein efficiency ratios of 1.11 and 1.25 for 70% rye bread fed at 6.0 and 6.4% levels of protein, while a whole-meal rye bread (9.7% protein) and a bread made from 82% rye flour (8.6% protein) gave protein efficiency ratios of 1.29 and 1.75 respectively. A white wheat bread fed at 8.6%

[2] H. M. Chemical Company, Ltd., Los Angeles, Calif.

and 10.4% levels of protein gave ratios of 0.81 and 1.09, respectively. Kon and Markuze used two to three animals per group, for a period of about 30 days, and a factor of 6.25 to convert nitrogen to protein. The level of protein in the diets was not constant. Because of the variable protein content they stated, "While our figures indicate that rye breads in general are superior, as far as the biological value of the protein goes, to wheat products, and that darker rye breads made from less refined flour are in turn superior to lighter varieties, we do not feel entitled to draw definite conclusions from the results of these tests."

An analysis of the amino acid composition of wheat and rye proteins, taken from the works of Baumgarten *et al.* in 1946 (1) and Horn *et al.* in 1950 (3) and recalculated to 16% nitrogen, showed that, with the exception of lysine, the percentages of essential amino acids in wheat and rye proteins are quite similar. The percentage of lysine in rye protein (3.3%) was higher than the 2.5 (1) and 2.6% (3) for wheat, but still much lower than the 7.1% in milk (3).

Table I shows the effects of supplements of nonfat dry milk and of lysine on the protein quality of rye bread ingredients and of rye bread and gives a statistical analysis of the differences between groups.

The addition of 6% nonfat dry milk significantly improved the protein efficiency both of rye water bread and of the unbaked ingredients, the increases being 9 and 11%, respectively. This is not so great as was found for whole-wheat bread under similar conditions (6), where the increases were 36 and 31% respectively, for the bread and for the unbaked ingredients. Sure (7) found that the addition of 0.25% l-lysine increased the protein efficiency ratio of whole-wheat flour 58% but increased that of rye flour only 9%. If lysine is the major contribution of nonfat dry milk, then the increase for rye bread might not be expected to be so large as for wheat bread.

The milk sample used had been analyzed for lysine, and a quantity of l-lysine equivalent to that in 6% nonfat dry milk was added to the unbaked ingredients without milk and incorporated in the water bread. Table I shows that this amount of lysine increased the protein efficiency 21% both for the bread and for the unbaked ingredients. The differences between groups receiving the lysine and the nonfat milk supplements were significant at the 5% level for the bread and at the 10% level for the unbaked ingredients (Table I). The body weight increases of the animals on the lysine supplements were only slightly greater than of those eating the rye milk bread and unbaked ingredients.

In a similar experiment with whole-wheat bread and unbaked in-

TABLE I

INFLUENCE OF BAKING AND OF LYSINE AND OF NONFAT DRY MILK ON THE QUALITY OF THE PROTEIN IN RYE BREAD
(Diets contained 1.42 ± 0.04% nitrogen. Experimental period, 5 weeks)

Group No.	Type of Ration	No. of Rats	Gains in Body Weight		Total Food Intake	Total Nitrogen Intake	Protein Nitrogen Efficiency [b]				Total Protein Intake (N × 5.83)	Protein Efficiency [c]		
			Amount	Increase			Amount	Standard Deviation	Comparison Group No.	"t" Value		Amount	Standard Deviation	Increase
			g.	%	g.	g.	g.				g.			%
I	Rye water bread	11	46.3	...	291	4.02	11.49	±0.94	...	...	23.4	1.97	±0.16	...
III	Rye bread containing 6% nfdm [a]	11	64.4	39	346	5.02	12.76	±0.91	I	3.24**	29.8 [d]	2.15	±0.15	9
V	Rye bread containing 0.166% lysine [a]	11	66.0	42	334	4.74	13.94	±1.51	I III	4.59** 2.22*	27.6	2.39	±0.26	21
II	Rye water bread, unbaked ingredients	11	61.1	...	332	4.82	12.63	±0.84	I	3.02**	28.1	2.17	±0.14	...
IV	Rye water bread, unbaked ingredients + 6% nfdm [a]	12	78.6	29	390	5.54	14.26	±1.27	II III	3.63** 3.26**	32.9 [d]	2.40	±0.21	11
VI	Rye water bread, unbaked ingredients + 0.166% lysine [a]	11	83.0	36	375	5.44	15.24	±1.39	II IV V	5.33** 1.75 2.10*	31.7	2.61	±0.24	21

[a] Flour basis.
[b] Expressed as gains in body weight per g. of nitrogen intake.
[c] Expressed as gains in body weight per g. of protein intake.
[d] N × 5.93.

gredients, the differences between groups receiving 0.166% lysine and 6% nonfat dry milk were not significant (t values of 0.49 and 0.29).

The same sample of nonfat dry milk was used in both the rye and the wheat experiments. The dry milk had analyzed 2.77% lysine from an acid hydrolysate, but 1.9% from an enzyme hydrolysate. The former value was used in calculating the amount of lysine to be added. The enzyme hydrolysate may give a lysine value for nonfat dry milk which is more in accord with the use of milk by the rat, or the free lysine may be better utilized by the rat than is the lysine from nonfat dry milk when combined with rye meal.

The relationships between essential amino acids within a single protein may be determined by computing the ratio of each acid with tryptophane. The amino acid balance of different proteins can thus be compared. The lysine to tryptophane ratios, taken from the data of Baumgarten (1) and Horn (3), are 5.5 and 4.1 for rye protein, 2.1 and 2.6 for wheat, and 6.4 for milk. Thus, the ratio for rye is about twice that for wheat and about 75% that of milk. This ratio may control protein efficiency to some extent, in which case the addition of 6% nonfat dry milk to rye or wheat would change the ratio less than would the addition of lysine alone.

Effect of Fermentation and Baking. The protein nitrogen efficiency ratios were lower for the baked breads than for the unbaked ingredients – 9, 12, and 9%, respectively, for the water, milk, and lysine breads. This loss in protein quality in the fermenting and baking of bread is in agreement with the decreases observed in whole-wheat breads (6), where the losses ranged from 11 to 33%.

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DETERMINATION OF SULFHYDRYL GROUPS IN WHEAT FLOUR[1]

R. W. Kong, D. K. Mecham, and J. W. Pence

ABSTRACT

Amperometric titration at a rotating platinum electrode with either mercuric chloride or silver nitrate appeared preferable to *o*-iodosobenzoate titration for estimation of sulfhydryl groups in wheat flour. Critical comparisons showed the amperometric methods to be easily reproducible, more precise, and probably more accurate.

Trypsin digestion caused the total flour protein to become soluble and to remain soluble during amperometric titration with the heavy metal ions. Titration values for dilute buffer extracts of untreated and trypsin-treated flours showed sulfhydryl groups probably to be absent from gluten. The experimental binding of sulfhydryl groups in flour extracts or digests with N-ethyl maleimide, removing the residual reagent with added crystalline bovine plasma albumin, and titrating the excess albumin with mercuric chloride showed that extracts or trypsin digests of flours contained little, if any, free cysteine or small sulfhydryl-containing peptides.

Biochemists working in many fields have directed a great deal of attention during the past two decades to the quantitative determination of sulfhydryl groups in proteins and peptides. The problem seems to be particularly difficult for the cereal chemist, because this very reactive group occurs at such low levels in wheat flour yet may be of great possible importance in mechanisms which are intimately associated with quality manifestations of flours and doughs.

Perhaps the most elaborate efforts to estimate sulfhydryl groups quantitatively in flour were those of Nordin and Spencer (19) with an adaptation of the *o*-iodosobenzoate method originally devised by Hellerman, Chinard, and Ramsdell (10). Holme and Spencer (13) later applied a titrimetric version of this method to flours and obtained good agreement with results by the parent method. Cuendet *et al.* (6), however, obtained much higher values with the titration procedure and expressed dissatisfaction with the method as used. A diffent method gave Sullivan (25) much lower results for the soluble proteins in flour than reported by either Cuendet *et al.* (6) or Spencer's group (13, 19). This method was the specific amperometric titration of sulfhydryl groups with mercuric chloride at a rotating platinum electrode, as described by Kolthoff, Stricks, and Morren (16).

Perhaps the principal difficulty in application of this amperometric

[1] Manuscript received July 23, 1956. Presented at the annual meeting, New York, May 1956. Contribution from Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, California.

method to gluten and flour is to keep the proteins, especially gluten, in solution during the mercurimetric titration. The present paper describes procedures devised to solubilize and to keep in solution the total protein of flour by means of tryptic digestion prior to amperometric titration with mercuric chloride. Comparisons were then made of the suitability of three methods for determining the sulfhydryl groups in flour. These methods were the iodosobenzoate, amperometric titration with mercuric chloride, and amperometric titration with silver nitrate, a new procedure for the latter method having recently been described by Benesch, Lardy, and Benesch (4).

Materials and Methods

Titrations with *o*-iodosobenzoate were conducted as described by Holme and Spencer (13). A measured amount of standard sodium *o*-iodosobenzoate solution was added to flour-water slurries (1:4), flour extracts and digests, or solutions of purified proteins, allowed to react for 3 minutes, and then centrifuged, in the case of flour slurries. A known excess of standard thiosulfate was then added to aliquots of the supernatant, just prior to acetic acid and potassium iodide. The residual thiosulfate was then titrated to a starch end point with a standard iodine solution.

Amperometric titrations by the method of Kolthoff, Stricks, and Morren (16) were conducted semiautomatically with a Leeds and Northrup Electrochemograph.[2] Small increments of titrant were added at regular intervals so that the chart tracing of the current could be used directly for graphical determination of the end points. Because optimal results for cysteine or small sulfhydryl-containing peptides require conditions different from those for proteins, the following procedure was employed to permit accurate estimation of sulfhydryl groups in both types of molecules simultaneously. Aliquots of solutions for analysis (10 ml. of a flour extract, for example) were first treated for 5 to 10 minutes at pH 7 with an excess of N-ethylmaleimide (1.0 ml. of a 0.001 *M* solution for 10 ml. of flour extract) to react specifically with the free sulfhydryl groups (9, 24). Excess N-ethylmaleimide was next bound with a known amount (100 mg. for 10 ml. of flour extract) of added crystalline bovine plasma albumin of known titer. The residual unreacted sulfhydryl groups of the plasma albumin were then determined by amperometric titration with mercuric chloride (0.001 *M*) under the conditions specified for protein materials (16). Extractions and titrations were conducted under a nitrogen atmosphere.

[2] Mention of trade names or equipment does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

Amperometric titrations with silver nitrate were made by the method of Benesch, Lardy, and Benesch (4). In this method tris (hydroxymethyl) aminomethane is used both as a complexing agent to protect silver ion from halides or other precipitating agents and to buffer the solution being titrated. Thus, neutral aqueous solutions may be used with or without denaturing agents, such as urea, for determination of both free and masked sulfhydryl groups.

Aqueous or dilute buffer extracts of flour for amperometric titrations were prepared by using a ratio of 4 ml. of liquid for each g. of flour and shaking or stirring the mixture for 1 hour. Trypsin digestion was accomplished with 200 mg. of trypsin (Fairchild) for each 25 g. of flour in 100 ml. of pH 7, 0.1 *M* phosphate buffer. Nitrogen was bubbled through the flour sample, the buffer, and the mixture of the two for 10 minutes each; a few drops of toluene were added; the container was tightly stoppered; and the mixture was shaken gently overnight at room temperature. By this digestion procedure all of the nitrogenous material of flours became soluble and remained soluble during titrations with either silver or mercury ions. Controls without trypsin were also usually included. Recoveries of sulfhydryl groups of proteins of known thiol content thus treated with the enzyme furnished fair assurance that no serious destruction of sulfhydryl groups had occurred. For instance, up to 92% of the sulfhydryl groups of bovine serum albumin added to flour before trypsin digestion was recovered. However, such recoveries ranged down to 70%, indicating that in some digests small amounts of sulfhydryls could have been derived from gluten without detection because of simultaneous decreases in values for all components of the mixture. In the absence of trypsin 90% or more of the sulfhydryl groups of flour extracts or of flour extracts containing added serum albumin was consistently recovered after standing overnight.

The flours used in these experiments were experimentally milled, straight-grade flours from pure-variety lots of wheat of different type and protein content. The wheat albumin and globulin preparations were from those described previously (21, 22). The crystalline bovine plasma albumin was Armour's Lot No. P67502. The crystalline ovalbumin was a laboratory preparation of high purity. Other materials and reagents were commercial preparations.

Results and Discussion

Analytical Comparison of Methods. Sulfhydryl values obtained by the three methods for some crystalline proteins and a commercial preparation each of glutathione and cysteine hydrochloride are in Table I.

TABLE I

SULFHYDRYL CONTENTS, AS EQUIVALENTS OF –SH PER MOLE, OF VARIOUS MATERIALS AS DETERMINED BY THE THREE ANALYTICAL METHODS

PREPARATION	ANALYTICAL METHOD			LITERATURE OR THEORETICAL VALUE
	Iodosobenzoate (13)	Hg + + Titration (16)	Ag + Titration (4)	
Bovine plasma albumin	1.6	0.74	0.68	0.67 (4, 14)
Ovalbumin	5.5	2.85		2.90–3.90 (4, 8, 11, 17)
Reduced glutathione	1.05	0.98[a]	0.98	1.00
Cysteine hydrochloride	0.92	0.98[a]	1.01	1.00

[a] Obtained by use of N-ethylmaleimide and excess plasma albumin.

Each of the methods gave satisfactory results with freshly prepared solutions of cysteine and glutathione, although the values for cysteine by the mercurimetric method and the idosobenzoate method were slightly lower than desirable. Both amperometric methods gave good agreement with literature values for bovine plasma albumin, but the iodosobenzoate result was definitely high. Likewise, with ovalbumin the amperometric results fitted at the lower end of the range of values found by other workers, but the result with iodosobenzoate was higher than most values reported for this protein. It should be mentioned that the value obtained with iodosobenzoate varied with the amount of reagent used for a given sample weight. This behavior in the case of flour extracts and slurries is discussed in more detail below.

Table II shows results obtained by the three analytical methods for the sulfhydryl contents of material extracted from a flour (Baart variety) by 0.1 *M*, pH 7 phosphate buffer with and without trypsin. The percentages of total flour nitrogen extracted were 99.2 and 16.8, respectively. These values are typical of those obtained with and without trypsin digestion. A replication of the entire experiment with another flour (Lee variety) gave results by the three sulfhydryl methods that were very close to those for the Baart flour and confirmed the lack of significant differences between values with and without trypsin.

TABLE II

SULFHYDRYL CONTENT OF MATERIAL EXTRACTED FROM FLOUR WITH AND WITHOUT TRYPSIN AS DETERMINED BY THE THREE ANALYTICAL METHODS

METHOD	EQUIVALENTS –SH/10^6 g. FLOUR	
	Without Trypsin	With Trypsin
Iodosobenzoate[a]	0.7	0.7
Hg + + titration	0.18	0.21
Hg + + titration in 6 *M* urea	0.24	0.26
Ag + titration	0.17	0.17
Ag + titration in 8 *M* urea	0.31	0.31

[a] 10 g. flour, 30.2 ml. water, and 1.8 ml. 0.0213 *N* sodium-iodosobenzoate solution, as used by Holme and Spencer (13). See also Fig. 1.

The values obtained with iodosobenzoate are only about half as large as those reported by Nordin and Spencer (19) and Holme and Spencer (13) for somewhat similar extracts of flour (without trypsin) and only about one-fifth as large as values obtained by Cuendet *et al.* (6) with Holme and Spencer's adaptation of the method. The range of these values by different investigators seems larger than can be accounted for by differences in flours used.

Two obvious sources of error in this method, namely, adsorption or reaction of iodosobenzoate or liberated iodine with starch or protein during the extraction and titration, were thoroughly studied by Nordin and Spencer (19), and seemingly adequate precautions were devised for them. Nevertheless, Hellerman *et al.* (10), in developing the method originally, particularly cautioned against use of excess iodosobenzoate when applying the method, since iodosobenzoate was known to be able to oxidize sulfhydryl groups as far as sulfonic acid under suitable conditions. Therefore, because the Holme and Spencer (13) method might naturally be applied with a fixed amount of iodosobenzoate for all flours and because flour is known to contain other oxidizable materials, an experiment was made to determine what effect an excess of iodosobenzoate might have on results obtained with the method as used in the present study. Examples of other oxidizable materials in flours might include pentosans (2) and lipid materials (5).

The values plotted in Fig. 1 were obtained by adding different amounts of iodosobenzoate to flour slurries and to aliquots of an extract of flour after centrifugation. The vertical dashed line represents the amount of reagent used by Holme and Spencer (13). Clearly,

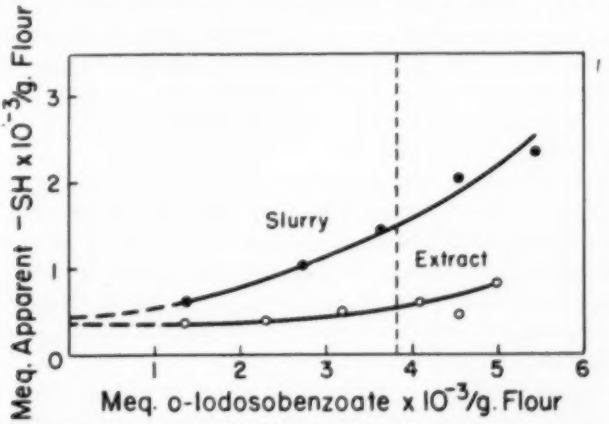

Fig. 1. Comparisons of "apparent" sulfhydryl values obtained for a flour slurry and an extract of the flour when varying amounts of o-iodosobenzoate were employed in the determination. Vertical dashed line indicates amount of reagent used by Holme and Spencer (13).

this is a large excess for both the slurries and the extract, and larger or smaller amounts would lead to different estimates of the "apparent" sulfhydryl contents of the samples. The value obtained for the flour slurry with the recommended amount of reagent agrees well enough with values reported by Nordin and Spencer (19) and Holme and Spencer (13) for similar materials—i.e., 1.5 Meq. $\times 10^{-3}$ per g. of flour vs. 1.2 and 1.34 Meq. $\times 10^{-3}$, respectively. Values for the extract are much lower, however, and are less subject to what appear to be nonspecific reactions. Extrapolation of these curves gives almost the same value for both slurry and extract and a value nearer those shown in Table II for the amperometric methods.

Reaction with oxidizable material in flours or flour extracts, other than sulfhydryl groups, and oxidation of sulfhydryl groups beyond the disulfide stage thus seem to be inherent disadvantages in the iodosobenzoate method. Holme and Spencer (13) noted that bromate and persulfate had little effect on sulfhydryl groups extracted from flour. Yet persulfate (1), if not bromate (12), rapidly disappears from flour-water doughs. Cookson and Coppock (5) recently called attention to earlier observations that oxidizing flour improvers produces oxidative changes in natural flour lipids. Presumably a portion at least of the lipid material in flour could be extracted by dilute salt solutions—phospholipids or lipoproteins, for instance. Serious doubts, therefore, might be raised concerning the specificity of iodosobenzoate as a reagent for determination of sulfhydryl groups in flour.

Amperometric methods, on the other hand, are subject to certain errors such as multivalent reactions between mercuric ions and sulfhydryl groups (16), dissociation of silver mercaptides (16), and sulfhydryl artifacts when ammoniacal or alkaline buffers are used (4, 15). Until such factors are shown to make the methods inappropriate for wheat flours, however, one may reasonably assume that the specific procedures the authors of these methods (4, 16) have worked out effectively guard against such errors.

All the above considerations plus general findings, reviewed by Olcott and Fraenkel-Conrat (20), that mercurial reagents are more specific for sulfhydryl groups than oxidizing agents, lead to the conclusion that amperometric titration is preferable for sensitive and accurate determination of the minute levels of sulfhydryl groups in wheat flour. Amperometric methods, furthermore, appear to be easily reproducible and have less subjective end points.

Sulfhydryl Contents of Gluten and Soluble Proteins. The analytical results shown in Table II illustrate another interesting and controversial point encountered in the present study. The apparent absence

of sulfhydryl groups in the insoluble or gluten proteins of the flours when brought into solution with trypsin conflicts with observations of De Lange and Hintzer (7), Matsumoto (18), and Baker, Parker, and Mize (3). These authors report values for the sulfhydryl contents of glutens determined by polarographic, amperometric, and colorimetric (ferricyanide-nitroprusside) methods, respectively. Repeated determinations with several flours in the present study failed to reveal, with any consistency, more than small amounts of sulfhydryl material in the large amounts of protein solubilized by trypsin treatment. Furthermore, these small increases in sulfhydryl content could have been due to globulin proteins not completely soluble in the dilute buffer solutions until after treatment with trypsin. Part of the sulfhydryl groups present in extracts and digests of flours seems to be masked or slow to react with titrating agents, as shown by the increases in titration values when urea is present in the solutions used for Table II. This increase with urea, however, was not found to be consistent when further comparisons were made.

While De Lange and Hintzer (7) took precautions to remove soluble proteins from gluten before analysis, they used ammoniacal solutions for dispersing the gluten for polarography. Ample evidence exists (4, 15) that ammonia and alkaline conditions, in general, are apt to create sulfhydryl artifacts and give erroneous results. Disulfide groups in many proteins are well known to be very sensitive to high pH values, breaking down to produce sulfhydryl and sulfenic acid groups (20). Matsumoto (18) also used ammonia and alkaline buffers, but whether or not he used purified glutens was not clear. Baker, Parker, and Mize (3) gave values for purified glutens, but their titration mixture included potassium cyanide which is known to reduce disulfide groups to produce sulfhydryl groups (20). In the absence of further evidence to the contrary, it appears doubtful that sulfhydryl groups reported by these workers actually existed in the native insoluble, or gluten, proteins of flour.

Adequate removal of soluble proteins from gluten is an important consideration, because studies on albumin and globulin proteins of flour have shown that unless precautions were taken, soluble proteins were incompletely removed from gluten during preparation (23). Gluten as ordinarily obtained by washing retains half or more of the soluble proteins originally present in the flour (23), and thus could contain appreciable amounts of sulfhydryl groups as impurities. Table III shows that flour albumins and globulins contain significant amounts of sulfhydryl groups.

Only slight differences are noted among the sulfhydryl contents of

TABLE III

SULFHYDRYL CONTENTS OF SOLUBLE WHEAT PROTEINS AS DETERMINED BY AMPEROMETRIC TITRATION (MERCURIC CHLORIDE METHOD)

PREPARATION	SOURCE	EQUIVALENTS -SH PER 10^6 G.[a]
Globulin No. 1	Wheat germ	0.12
No. 2	Wheat germ	0.13
No. 3	Commercial flour	0.12
Albumin No. 1	Comanche flour	0.05
No. 2	Thatcher flour	0.07
No. 3	Pentad (durum) flour	0.10
No. 4	Hymar flour	0.07

[a] Expressed in this manner because of unknown molecular weight.

the globulin preparations, despite previously determined variations in their proportions of individual components (22). Greater differences in sulfhydryl content appear to occur among the albumin preparations, but estimates of their component composition are not available. Differences in sulfhydryl content among preparations of soluble proteins do not necessarily reflect similar variations in sulfhydryl contents of the original flours, because the preparative procedures are known to alter the ratios of individual albumin or globulin components during purification (21, 22).

Sulfhydryl Contents of Different Flours. Analytical results obtained by the amperometric titration with four flours of varying type and protein content are given in Table IV. Neither trypsin digestion nor the use of N-ethylmaleimide for the mercurimetric titration was employed for these samples, since extensive comparisons with and without N-ethylmaleimide indicated that flours or their trypsin digests contained little, if any, free cysteine or small sulfhydryl-containing peptides. The values shown are averages of two to four separate determinations for each flour by each method. The greatest range of

TABLE IV

SULFHYDRYL CONTENTS OF FLOURS AS DETERMINED BY AMPEROMETRIC TITRATION ON MATERIAL EXTRACTED BY pH 7, 0.1 *M* PHOSPHATE BUFFER

VARIETY	PROTEIN CONTENT[a]	EQUIVALENTS -SH PER 10^6 G. FLOUR	
		Mercuric Chloride	Silver Nitrate
	%		
Red Chief	12.2	0.20	0.22
Pentad (durum)	11.6	0.26	0.30
Thatcher	11.4	0.18	0.23
Baart	11.3	0.20	0.22

[a] At 14% moisture.

values for single flours by the mercuric chloride titrations was ±0.04 equivalents and was ±0.05 by the silver nitrate titration. Values by the two methods therefore may not be significantly different, although the consistency of the differences suggests that the silver nitrate results are higher. In general, the silver nitrate method is more convenient to use because the same analytical conditions are used for all types of sulfhydryl-containing material. The mercuric chloride titrations, on the other hand, gave more satisfactory curve tracings for end-point determination. Urea produced no consistent increase in titration values by either method.

The values in the table are higher than reported by Sullivan (25) for a distilled water extract of flour (0.10 equiv/10^6 g. flour), although this author pointed out that higher values were obtained when salt solutions were used for extraction of the flour. Increasing the concentration of phosphate for extraction of the soluble proteins from flours in the present experiments dissolved more of the flour nitrogen, but did not increase the sulfhydryl values significantly.

As yet, the number of flours analyzed by amperometric methods (four) is too small to assign any particular significance to the moderate variation in sulfhydryl content. A survey of the sulfhydryl contents of a series of typical flours in an effort to discover any relationships between sulfhydryl contents and baking quality or other characteristics will soon be undertaken.

Acknowledgments

The authors are indebted to Dr. Wilfred Ward of this Laboratory for assistance with the silver nitrate amperometric titrations referred to in this study, and to Mr. E. D. Ducay for the crystalline ovalbumin.

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STUDIES ON THE SOLUBLE DEXTRIN FRACTION AND SUGAR CONTENT OF BREAD BAKED WITH ALPHA-AMYLASE FROM DIFFERENT SOURCES[1]

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ABSTRACT

Bread was made using a range of concentrations of alpha-amylase from malted wheat flour, a fungal source (*Aspergillus oryzae*), and a bacterial source. The amount of soluble dextrin extracted from the bread crumb 1 hour after baking increased with each increment of malted wheat flour and bacterial alpha-amylase. Fungal alpha-amylase did not affect the amount of extractable soluble dextrins because of its low thermostability. The concentration of malted wheat flour and fungal enzyme did not have an appreciable effect on the average chain length of the dextrin. The average chain length of the soluble dextrins extracted from bread crumb of bread made with bacterial amylase was several times greater than those obtained from bread made with malted wheat flour and fungal enzymes, and depended on the concentration. High concentration of bacterial alpha-amylase reduced the average chain length of the soluble dextrin compared to low concentrations.

The residual glucose in bread crumb increased rapidly with increasing concentrations of bacterial amylase but less rapidly with increasing concentrations of cereal or fungal alpha-amylase. The residual maltose in bread crumb increased much less with increasing quantities of fungal than with comparable quantities of cereal or bacterial alpha-amylase.

Enzymatic modification of starch in baking produces end products which affect the quality of bread. This modification of starch by amylase action is one practical way to alter the rate of crumb firming, the extent of which depends on the amount and thermostability of the alpha-amylase present (9, 13). If the concentration of alpha-amylase or its thermostability is sufficiently high to completely eliminate crumb firming, a gummy crumb will result (10). Because of their relatively low thermostability, cereal and fungal alpha-amylase supplements have been found to provide a practical means for retarding the firming of bread crumb. Jackel, Schaeder, and Schultz (8) have shown that bacterial alpha-amylase continues to degrade starch during bread storage. Walden (14) studied the action of wheat amylases on starch under conditions of time and temperature as they exist during baking. He showed that a high degree of starch conversion was produced by the action of alpha-amylase. In combination with beta-amylase, larger

[1] Manuscript received August 3, 1956. Cooperative investigations between the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Department of Flour and Feed Milling Industries, Kansas State College. Contribution No. 272, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan.

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amounts of maltose were produced and the average chain length of the dextrins was reduced. Walden (14) did not investigate fungal or bacterial amylases.

Miller, Johnson, and Palmer (10) compared cereal, fungal, and bacterial amylase preparations as supplements for breadmaking. They found that the firmness of the bread crumb, as well as the amount and character of the dextrin extracted from bread, was related to the thermostability of the enzymes used. An increase in concentration of the enzymes produced soluble dextrins with a somewhat larger degree of polymerization. The amount of extractable dextrin in bread also increased with increasing amounts of all amylases, but with bacterial amylase this increase was much greater than with fungal or cereal amylase. With the aging of bread the average molecular weight of the dextrins decreased, owing to insolubilization or crystallization of the larger dextrins.

Miller, Johnson, and Palmer (10) used a rather drastic extraction procedure to extract the soluble dextrins from bread crumb. They also used only the ferricyanide method (2) for the determination of the average chain length of the dextrin. The object of the present study was to determine the amounts of soluble dextrin, glucose, and maltose in bread when a mild extraction procedure was employed. The chain length of the dextrin was determined by two chemical methods. A range of concentrations of bacterial, cereal, and fungal enzymes was employed.

Materials and Methods

An unmalted commercial hard red winter patent flour with 0.41% ash and a protein content of 11.8% (14% moisture basis) was used in all experiments.

The enzyme supplements included a commercial malted wheat flour and two commercial enzyme concentrates, one of which, Diastase-33[4] (*Aspergillus oryzae*), was of fungal and the other, Rhozyme DX[4], of bacterial origin. The alpha-amylase activities of these preparations were 75, 5335, and 253 SKB units (12) per g., respectively. The beta-amylase activity in the flour or enzyme preparations was not determined. Flour is known to contain a large amount of this enzyme and the quantity contributed by the enzyme supplement is of no significance. The effect of any amyloglucosidase in the enzyme preparations also was considered to be negligible.

The baking experiments were carried out on a laboratory scale using a 70% sponge-dough procedure. The formula was as follows:

[4] Rohm and Haas Co., Philadelphia, Pa.

Ingredient	*Sponge*	*Dough*
	%	%
Flour	70	30
Yeast	2	...
Yeast food	0.5	...
Enzyme supplement	Variable	...
Sucrose	...	5
Sodium chloride	...	2
Nonfat dry milk	...	3
Shortening	...	3
Water	Variable	Variable

The sponges were fermented for 3 hours at 86°F. After mixing to optimum consistency, the doughs were given a 30-minute floor time, scaled to 18 oz., and given 20 minutes' rest before molding. The doughs were proofed at 94°F. for 55 minutes and baked for 28 minutes at 425°F. Sugars and dextrins were extracted from the crumb 1 hour after baking.

Preparation of Bread-Crumb Extract. The sugars from 100 g. of bread crumb were extracted by refluxing for 2.5 hours with 320 ml. of 95% ethyl alcohol. Considering the water in the crumb, the final extract contained 84% alcohol. The alcohol was removed from the supernatant by distillation under reduced pressure. The concentrate was then centrifuged and the supernatant was made up to 50 ml. with water. This solution was deionized (15), using 2 g. each of Dowex 50 and Duolite A-4 resin. After filtering, the liquid was stored in the refrigerator until chromatographic analyses were performed.

The alcohol extraction residue was extracted with 500 ml. of water for 2 hours at 30°C. The centrifugate was stored at −10°C. until the quantity and average chain length of the soluble dextrins could be determined.

Determination of Soluble Dextrin. Twenty milliliters of frozen bread extract were thawed rapidly and added to 100 ml. of 95% ethyl alcohol. The precipitate accumulating during 16 hours at 5°C. was collected in a tared Gooch crucible, washed with 10 ml. of 80% ethyl alcohol, dried at 130°C. for 2 hours, and weighed (3).

Determination of Average Chain Length of the Dextrins. The average chain length of soluble dextrins in the crumb of the bread obtained with the various enzyme supplements was determined in aliquots of the above-described aqueous dextrin solutions, by two different methods (1, 2).

1. *Periodate method.* Twenty-milliliter portions of a standard glucose solution, a blank, and the aqueous dextrin solution were added to separate 20-ml. portions of 0.25 *N* sodium metaperiodate plus 10 ml. of water and stored at 5°–6°C. in the dark. Five-milliliter aliquots

were removed at suitable intervals during a 5-day period and the excess metaperiodate was removed by allowing the reaction with 0.5 ml. of ethylene glycol to proceed in dim light for 10 minutes. Excess potassium iodide then was added followed by a slight excess of 0.01 *N* sodium thiosulfate which was back-titrated with 0.01 *N* iodine using starch as the indicator.

2. *Ferricyanide method.* Analyses for reducing groups were performed both before and after hydrolysis of the dextrin by autoclaving for 30 minutes at 15 lb. in the presence of 0.5 *N* sulfuric acid. Five-milliliter aliquots of a standard glucose solution, a blank, and the dextrin solution before and after hydrolysis were added to 10 ml. aliquots of 0.1 *N* alkaline ferricyanide in 50-ml. test tubes and mixed by swirling. The tubes were immersed in a vigorously boiling water bath for exactly 20 minutes. The contents were cooled immediately and rinsed into a flask with 25 ml. of acetic acid-salt solution. One milliliter of soluble starch-potassium iodide solution was added and the contents titrated with 0.1 *N* sodium thiosulfate.

Determination of Reducing Matter in Bread-Crumb Extracts. The presence of reducing matter other than reducing carbohydrates in the bread extract was determined by the iodometric method of Freilich (6). The reducing matter in 5 ml. of bread extract was allowed to react with 10 ml. of 0.01 *N* iodine solution containing 0.24 g. of potassium iodide. The excess iodine was determined by back-titration with 0.01 *N* sodium thiosulfate.

Estimation of Maltose and Glucose in Bread Crumb. Maltose and glucose in bread crumb were chromatographed by the method employed by Griffith and Johnson (7). The sugar spots were located with the use of aniline-diphenylamine (4) and estimated quantitatively by the method of Dubois *et al.* (5).

Results and Discussion

Grain and texture scores increased for bread made with low levels of alpha-amylase from cereal, fungal, and bacterial sources (Table I). Higher levels of these enzymes caused a reduction in the grain and texture scores but had little effect on loaf volume. The greater efficiency of bacterial amylase was caused by its thermostability (10). High levels of bacterial amylase were particularly detrimental to the grain and texture.

The data in Table I obtained in replicate experiments show that the amount of soluble dextrins extracted from the bread crumb increased with each increment of malted wheat flour and bacterial alpha-amylase. The fungal alpha-amylase increased the soluble dextrins very

TABLE I

EFFECT OF ALPHA-AMYLASE TYPE AND CONCENTRATION ON BREAD QUALITY AND AMOUNT AND AVERAGE CHAIN LENGTH OF SOLUBLE DEXTRINS PRODUCED IN BREAD

Enzyme	Alpha-Amylase[a]	Loaf Volume	Crumb Scores		Soluble Dextrin	Average Glucose Units in Dextrin	
			Grain	Texture		Periodate Method	Ferricyanide Method
	units	*cc.*	*%*	*%*	*% of bread crumb*		
Control	None	2450	80	80	1.5	9	9
	140	2790	90	90	2.2	8	10
Malted wheat flour	560	3000	85	90	3.1	9	11
	1120	2860	80	85	3.7	10	11
	140	2750	95	95	1.9	9	7
Fungal	560	2900	85	85	2.1	9	8
	1120	2950	80	80	1.9	7	10
	7	2600	90	90	2.8	13	12
Bacterial	35	2600	90	80	5.7	34	22
	140	2640	75	60	10.6	18	16

[a] SKB units per 700 g. flour (12).

little because it was inactivated before much of the starch became gelatinized and available to enzyme attack (10). The amount of soluble dextrins was increased markedly by the bacterial alpha-amylase because it was active for a relatively long time after the starch became gelatinized.

The average chain length of the soluble dextrins did not vary significantly as the amount of malted wheat flour and fungal amylase was increased. The lack of effect of increasing amounts of fungal and malted wheat flour alpha-amylase undoubtedly was due to the limited thermostability of these amylases. Malted wheat flour alpha-amylase, however, was thermostable enough to have an effect on the solubilization of the starch and the amount of dextrin which could be extracted under the mild conditions of these experiments. The highest level of bacterial amylase (140 SKB units) caused a decrease in the average chain length of the dextrin. It is assumed that this occurred because of its continued activity at elevated temperatures on the soluble dextrins as well as on the starch. This is in contrast with the longer chain lengths occurring when 35 SKB units were employed. The ferricyanide (2) and the periodate (1) methods gave comparable results for the average chain lengths.

The average chain lengths of the dextrins were not so long as those determined by Miller, Johnson, and Palmer (10). This might be due to the differences in the methods employed to extract the soluble dextrins, or to the presence of noncarbohydrate reducing matter. Testing the water extracts of the dextrins by an iodine titration method (6)

showed that the amount of reducing matter was negligible. Therefore, it appears that the mild extraction procedure used to obtain the water-soluble dextrins, compared to the drastic procedure used by Miller, Johnson, and Palmer (10), accounts for the lower calculated average chain lengths in this study. They also demonstrated an increase in the average chain length of the dextrin as the amount of malted wheat flour and fungal alpha-amylase was increased. The extraction procedure employed in the present experiments was too mild to bring out the effects observed in the earlier experiments (10). Nevertheless, the trends on the effect of alpha-amylase on the amount of extractable dextrin were confirmed.

It has been shown that the action of beta-amylase on starch produces only maltose, but hydrolysis with alpha-amylase produces glucose, maltose, and sugars of higher molecular weight (11). It also has been shown that there is a linear relationship between sugar added to the dough and the residual sugar in the crumb (7). Similarly, a relationship might be expected between the sugar produced by amylolysis during fermentation and baking, and the residual sugar of the bread crumb. However, account must be given to the utilization of sugar by the yeast during the fermentation and early stages of the baking process. Figure 1 indicates that an increase in the glucose content of the crumb was obtained with increased alpha-amylase. The increase in maltose was less with the fungal supplement because of the heat-inactivation

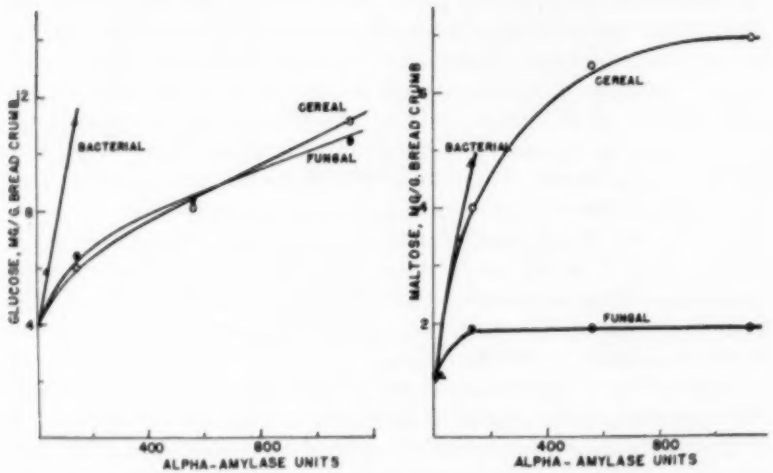

Fig. 1. Relationship between the quantities of glucose and maltose in bread crumb and alpha-amylase concentration (SKB units/700 g. of flour).

of the alpha-amylase early in the baking process. This indicates that the different alpha-amylase sources produced different amounts of maltose during the *baking* process.

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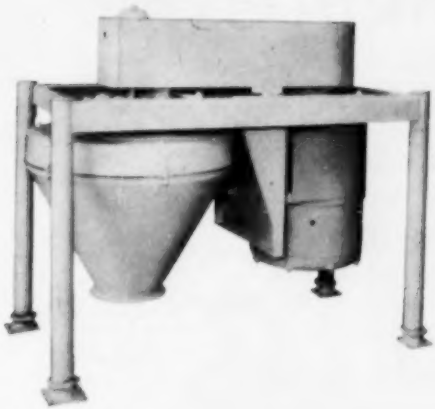

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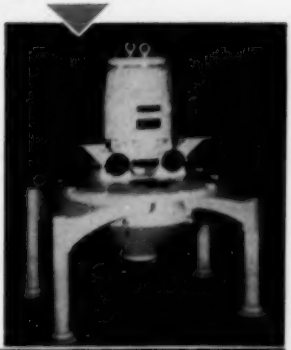

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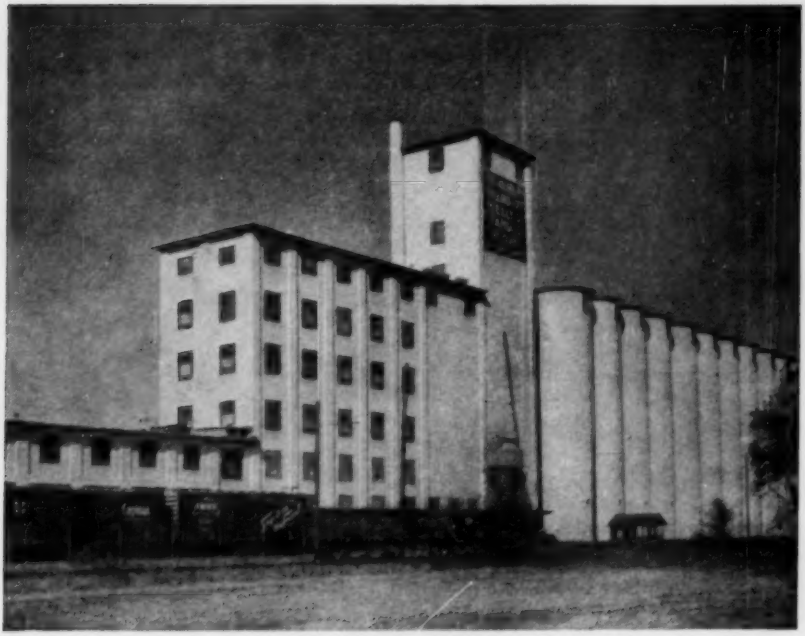

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